IDENTIFICATION, CHARACTERIZATION AND DEGRADATION STUDIES OF POLYCYCLIC AROMATIC HYDROCARBONS IN RECREATIONAL URBAN LAKES, ROAD DUSTS AND ROAD SIDE SOILS OF KUALA LUMPUR CITY, MALAYSIA

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THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY
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KUALA LUMPUR

2013

ABSTRACT

This study is on the distributions and concentrations of 16 polycyclic aromatic hydrocarbons (PAHs) in eleven surface water, suspended particulate matter (SPM) and surface sediments samples collected from two urban recreational lakes namely, Taman Jaya and Lake Perdanas in Kuala Lumpur city. The total PAH concentrations of Lake Taman Jaya and Lake Perdana ranged from 0.56- 1.19 µg L^{-1} and 0.32- 1.30 µg L^{-1} in surface water, 0.93- 2.80 µg g^{-1} and 1.21-2.92 µg g^{-1} in SPM, and 0.15– 0.23 µg g^{-1} and 0.01– 0.27 µg g^{-1} in surface sediments respectively. High molecular weight (HMW) PAHs dominates the distribution of PAHs suggesting a great influence of pyrogenic activities. The ratio of Phen/Anth to Flt/ Pyr suggests that the major sources of PAHs in Lake Taman Jaya and Lake Perdana were from both petrogenic and pyrogenic sources. The levels of PAHs in both lakes are relatively higher in surface waters and SPM, and lower in surface sediments, in comparison to those reported around the world. The calculation of TEQ^{carc} in surface sediments of both lakes shows that it was low compared to other previous studies worldwide.

On the other hand, studies on PAHs in road dust and road side soil samples of Kuala Lumpur city shows that road dust of the residential areas (9.25- 689 µg g^{-1} dw) were highly polluted with PAHs compared to the industrial (1.30- 33.2 µg g^{-1} dw) and commercial areas (23.3- 571 µg g^{-1} dw). Meanwhile, the results for road side soil samples shows that PAHs in commercial areas (15.7- 1593 µg g^{-1} dw) were more polluted with PAHs than the industrial (0.387- 36.5 µg g^{-1} dw) and residential areas (0.119- 180 µg g^{-1} dw). High molecular weight PAHs dominates studied areas with pair ratios calculation of Phen/ Anth to Flt/ Pyr shows that the major cause of these PAHs pollutions came from pyrogenic activities.

A study on identification of natural capability in degrading PAHs has also been conducted. A few isolated bacteria from termite fungal comb and contaminated road side soil have been selected and grouped for the degradation study. Among the nine single PAHs tested, only fluoranthene and pyrene shows positive sign of degradation against the applied bacteria consortium. One degradation product has been detected from degradation of fluoranthene by bacteria consortium isolated from termite fungal comb (1-butyl-8-methyl-1,8a-dihydronaphthalene) while two degradation products has been detected from degradation by bacteria consortium from road side soil (1-vinyl-9H-fluorene and 9-methylene-1-vinyl-9H-fluorene). Meanwhile, the degradation of pyrene by bacteria consortium from termite fungal comb and road side soil has produced three (1,10a-dihydropyrene, 9-ethyl-1-methyl-1H-phenalene and 2-ethyl-1-isopropyl-8-methyl-1,2,3,4,4a,8a-hexahydronaphthalene) and four (1,10a- dihydropyrene, 9-ethyl-1-methyl-1H-phenalene, 2-ethyl-1-isopropyl-8-methyl-1,2,3,4,4a,8a-hexahydronaphthalene and 7-butyl-1-methyl-1,2-dihydronaphthalene) degradation products, respectively. Overall, the degradation of fluoranthene and pyrene by the selected bacteria consortium increased with time but the degradation process is considered slow. Degradation by bacteria consortium from contaminated road side soil was found to be more effective than bacteria consortium from termite fungal comb.

ABSTRAK

Kajian ini adalah mengenai taburan dan kepekatan kandungan 16 jenis hidrokarbon aromatik polisiklik (PAHs) di permukaan air, bahan terampai, dan sedimen dari dua tasik yang terletak di tengah- tengah Bandaraya Kuala Lumpur (Tasik Taman Jaya dan Tasik Perdana). Kepekatan keseluruhan PAHs yang direkodkan di Tasik Taman Jaya dan Tasik Perdana adalah dari 0.56 ke 1.19 µg L^{-1} dan 0.32 ke 1.30 µg L^{-1} untuk sampel permukaan air, 0.93ke 2.80 µg L^{-1} dan 1.21 ke 2.92 µg L^{-1} untuk sampel bahan terampai dan 0.15 ke 0.23 µg L^{-1} dan 0.01 ke 0.27 µg L^{-1} untuk sampel permukaan sedimen. PAHs dengan berat molekul tinggi mendominasi di kedua- dua tasik sekaligus menunjukkan bahawa PAHs di kawasan kajian berasal daripada aktiviti pyrogenik. Data pengiraan nisbah Phen/Anth kepada Flt/ Pyr menunjukkan bahawa sumber PAH di kedua- dua tasik adalah daripada kedua- dua aktiviti petrogenik dan pyrogenik. Jika dibandingkan dengan kajian kepekatan PAHs yang dilaporkan di seluruh dunia, kepekatan PAHs di kedua- dua tasik ini secara relatifnya adalah tinggi di permukaan air dan bahan terampai, dan rendah di permukaan sedimen. Walaubagaimanapun, data kiraan TEQ^{carc} bagi kedua- dua tasik ini adalah lebih rendah daripada kajian- kajian lain yang pernah direkodkan di seluruh dunia.

Kajian PAHs juga turut dijalankan terhadap sampel debu jalanraya dan tanah di tepi jalanraya di sekitar Bandaraya Kuala Lumpur dan hasil kajian menunjukkan bahawa debu jalanraya di sekitar kawasan perumahan (berat kering 9.25- 689 µg g^{-1}) mengandungi lebih banyak PAHs berbanding kawasan perindustrian (berat kering 1.30- 33.2 µg g^{-1}) dan kawasan komersil (berat kering 23.3- 571 µg g^{-1}). Sementara itu, kajian kepekatan PAHs terhadap sampel tanah dari tepi jalanraya menunjukkan bahawa kepekatan PAH di kawasan komersil (berat kering 15.7- 1593 µg g^{-1}) adalah lebih tinggi berbanding di kawasan industri (berat kering 0.387- 36.5 µg g^{-1}) dan di kawasan perumahan (berat kering 0.119-

180 µg g^{-1}). Dominasi PAHs dengan berat molekul tinggi dan keputusan kiraan nisbah Phen/Anth kepada Flt/ Pyr menunjukkan bahawa sumber utama pencemaran PAHs bagi kedua- dua jenis sampel iaitu sampel debu jalanraya dan tanah ditepi jalanraya adalah dari aktiviti pyrogenik.

Selain daripada kajian terhadap taburan dan kepekatan PAHs, kajian untuk mengenalpasti kebolehan sumber semulajadi dalam mendegradasi PAHs juga turut dijalankan dan dilaporkan. Di dalam kajian ini, beberapa spesis bakteria yang telah diekstrak keluar daripada dua jenis sampel iaitu sampel sarang anai- anai tanah dan sampel tanah dari tepi jalanraya telah digunakan melalui pembahagian kepada beberapa kumpulan. Ujian degradasi terhadap sembilan jenis PAHs telah dijalankan menggunakan kumpulan bakteria ini dan hanya dua daripada sembilan PAHs ini menunjukkan kesan positif. PAHs yang telah dikenalpasti bertindak balas ini adalah fluorantena dan pirena. Sejenis produk degradasi telah dikenalpasti dihasilkan dari proses degradasi fluorantena oleh kumpulan bakteria dari sarang anai- anai tanah (1-butil-8-metil-1,8a-dihidronaftalena) manakala kumpulan bakteria dari tanah pula telah dikenalpasti menghasilkan dua jenis produk (1-vinil-9H-fluorena dan 9-metilena-1-vinil-9H-fluorena) dalam ujian degradasi dengan jenis PAH yang sama. Sementara itu, kumpulan bakteria dari sarang anai- anai dan tanah tercemar masing- masing turut dikenalpasti menghasilkan tiga (1,10a- dihidropirena, 9-etil-1-metil-1H-phenalena dan 2-etil-1-isopropil-8-metil-1,2,3,4,4a,8a-heksahidronaftalena) dan empat (1,10a- dihidropirena, 9-etil-1-metil-1H-phenalena, 2-etil-1-isopropil-8-metil-1,2,3,4,4a,8a-heksahidronaftalena and 7-butil-1-metil-1,2-dihidronaftalena) produk dari kajian degradasi mereka terhadap pyrene. Keseluruhannya, peratusan degradasi fluorantena dan pirena oleh kumpulan bakteria terpilih ini semakin tinggi dengan peningkatan masa walaupun prosesnya adalah perlahan. Kumpulan bakteria daripada tanah yang tercemar

didapati mendegradasi fluorantena dan pirena dengan lebih efektif daripada kumpulan bakteria dari sarang anai- anai tanah.

ACKNOWLEDGEMENT

I am grateful to Allah for guiding me to complete this PhD program. Indeed, without His Help and Will, nothing is accomplished.

I wish to express my sincere gratitude to my supervisor and co- supervisor, Prof. Dr Mhd. Radzi Abas and Associate Professor Dr. Nor Kartini Abu Bakar for their excellent guidance, caring, and patience. Special thanks to University of Malaya for giving me an opportunity to undergo this PhD program and Yayasan Khazanah for their financial support, leadership skills and a “family” to lean on throughout this education journey.

I would like to thank Dr. Tay Kheng Soo for his help in guiding my research for the past several years and my other lab mates namely Chin Yuk Ping, Aliaa Diyana, Dr. Mohd Shahrul Mohd Nadzir, Noorashikin and Farhana for their help and co- operation. To my best friends Fathiah Asmadi, Norliyana Zairudin, Norfarisah Ahmad Saufian, Norashikin Sallih Udin, Fadzillah Mansor, Mohd Shaufi Sokiman and all my friends, thank you for your time listening to me and your support along this journey. My acknowledgement also goes to Mr. Asha’ari Kadir and all my ex- collogues for their support.

Last but not least, my deepest gratitude goes to my family especially my parents, Hj. Mohd Radzi A. Rahman and Hajjah Siti Rohani Hj. Bidin for their love, continuous supports, believe and encouragement all this time. To my sisters Nurayuni and Juliana Seman, from the bottom of my heart, thank you so much for always being there for me whenever I’m in need and encouraging me when I need it the most. For my lovely other family members, especially Saiful Bahri, Yuhanis, Muhammad Maliki, Mohd Samsul Najmi, Muhammad Warith Hakimi and Muhammad Muqrie, thank you so much for everything. It would not have been possible for me to write this doctorate thesis without the help and support of these kind people around me.

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ABBREVIATIONS

Ace	Acenaphthene
A_{contm}	Amount of Contamination
ACS	American Cancer Society
Acy	Acenaphthylene
Amt	Amount
Ant	Anthracene
$Area_{ext}$	Area of the External Standard
ATSDR	Agency for Toxic Substance and Disease Registry
BaA	Benzo[a]anthracene
B[*a*]Peq	Benzo[a]pyrene-Equivalent Concentration
BbF	Benzo[b]fluoranthene
BgP	Benzo[g,h,i]perylene
BkF	Benzo[k]fluoranthene
BaP	Benzo[a]pyrene
BW	Body Weight
C	Contaminant Concentration
CI	Chemical Ionization
Conc.	Concentration
$Conc_{contm}$	Contaminant Concentration
$Conc_{ext}$	Concentration of the External Standard
CPAH	Carcinogenic PAHs
CRM	Certified Reference Material
Crys	Chrysene
CSL	Chan Saw Lin
D	Dose

DbA	Dibenzo[a,h]anthracene
DCM	Dichloromethane
DOM	Department of Meteorology
DOS	Department of Statistic
dw	Dry Weight
EF	Exposure Factor
e.g.	Exempli Gratia/ For Example
EI	Electron Impact
ERL	Effective Range Low
ERM	Effective Range Medium
FDEP	Florida Department of Environmental Regulation
Flt	Fluoranthene
Flu	Fluorene
GC	Gas Chromatography
GC- FID	Gas Chromatography- Flame Ionization Detector
GC- MS	Gas Chromatography- Mass Spectrometry
HMW	High Molecular Weight
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
IDL	Instrument Detection Limit
IF	Inoculation Fluid
InP	Indeno[1,2,3- cd]pyrene
IR	Intake Rate
ISO	International Standards Organization
Kg	Kampung
KLCH	Kuala Lumpur City Hall

LLE	Liquid- Liquid Extraction
LMW	Low Molecular Weight
LRAT	Long Range Atmospheric Transport
Max	Maximum
MDL	Method Detection Limit
MeOH	Methanol
Min	Minimum
MSM	Mineral Salt Medium
na	Not Available
Naph	Naphthalene
NCI	National Cancer Institute
NOAA	National Oceanic and Atmospheric Administration
ONS	The Office for National Statistics
PAHs	Polycyclic Aromatic Hydrocarbons
PC	Principal Component
PCA	Principal Component Analysis
PEL	Probable Effect Level
Phen	Phenanthrene
PPR	Projek Perumahan Rakyat
Pyr	Pyrene
QC	Quality Control
R	Recovery
RF_{avg}	Average Response Factor
R.S.D.	Reproducibility in Standard Deviation
Sal	Salinity
S.D.	Standard Deviation

Sg	Sungai
SIM	Selected Ion Monitoring
SpC	Specific Conductance
SPE	Solid Phase Extraction
SPM	Suspended Particulate Matter
TDS	Total Dissolved Solids
TEF	Toxic Equivalency Factors
TEL	Threshold Effect Level
Temp	Temperature
TEQ^{carc}	Total Toxic Benzo[*a*]pyrene Equivalency
TIC	Total ion chromatography
Tof	Time- of- flight
TTDI	Taman Tun Dr. Ismail
Tur	Turbidity
USEPA	United State Environmental Protection Agency
UV	Ultra Violet
V_{binj}	Total Volume before Injection
V_{ext}	Volume of the Extract
V_{frc}	Volume of the Extract Used for Fractionation
V_{inj}	Injection Volume
W	Weight

Chapter 1

Chapter 1

General Introduction

1.1 General environmental conditions of Kuala Lumpur, Malaysia

Study areas were located around Kuala Lumpur City, the capital city of Malaysia which has a geographical coordinate between 3° 10' North latitude and 101° 42' East longitude. At this latitude and longitude, Kuala Lumpur or Malaysia as a whole has a tropical weather, influenced by monsoonal climate. Tropical climate here gives hot summer that is accompanied with high humidity level. But the weather in general in Malaysia is without extremities. Monsoon comes twice a year where one would occur from late May to September which known as Southwest Monsoon and the other from November to March which known as Northeast Monsoon. Northeast monsoon brings lots of downpour in Malaysia compared to the Southwest Monsoon. Southwest Monsoon does not cause that much rain and is generally dry. The amount of rainfall recorded in Kuala Lumpur for 2009, ranges from 136 to 424 mm and from 110 to 537 mm in 2010 (DOM, 2011).

With an area of 243 km^2 (94 sq mi), Kuala Lumpur is a residence of 1.7 million people (DOS, 2010) and the fastest growing metropolitan region in the country. High volume of population also leads to high density of vehicles. In Malaysia, the number of vehicle registered has increased from 13.8 million to 18.0 million between 2004 and 2008 (DOS, 2010).

Mostafa *et al*. (2009) mentioned that industrial wastes, traffic congestion and over-crowding in cities will lead to pollutants that significantly contribute to environmental damage and health problems. As the most industrialized and economically the fastest growing region in Malaysia, vehicular emission has been identified as its key pollution

sources (DOS, 2010; DOE, 2008). Other possible sources of pollution in Kuala Lumpur are industrial emissions, open burning and the occasional smoke particles from forest fires near Sumatra, Indonesia (Omar *et al*., 2002; Omar *et al*., 2006).

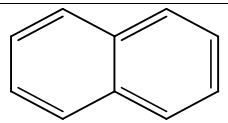
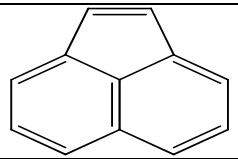
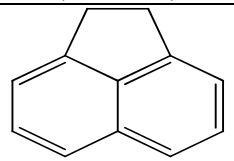
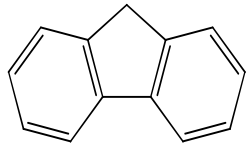
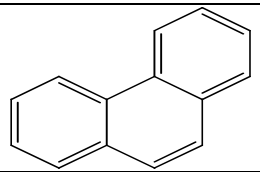
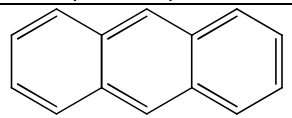
1.2 Polycyclic aromatic hydrocarbons (PAHs)

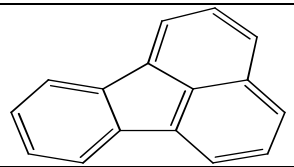
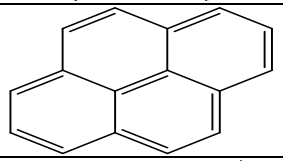
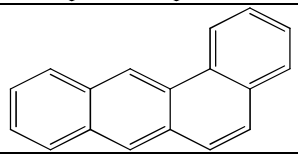
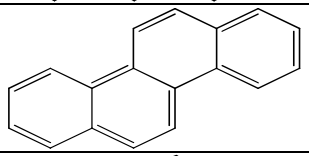
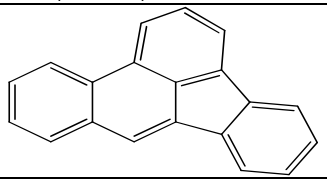
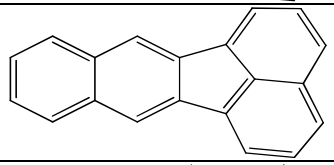
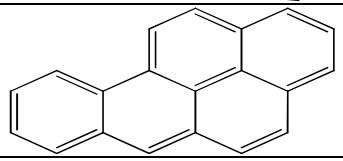
This study is focused on organic compounds known as polycyclic aromatic hydrocarbons (PAHs). PAHs are a group of compounds containing numerous hundred of individual compound with at least two condensed rings. It exists and normally detected in the atmosphere, water sources, living organisms and soils which includes dust. PAHs are characterized by multiple aromatic rings with various organic constituents, number of connected aromatic rings, size of the aromatic rings as well as the functional groups substituted onto the aromatic rings.

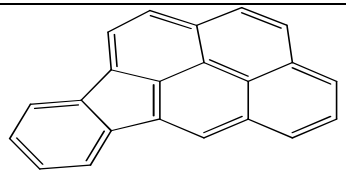
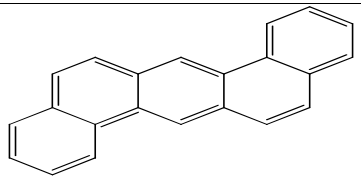
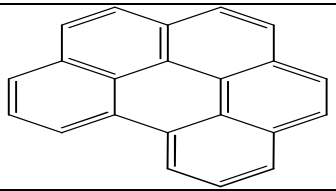
1.2.1 Chemical and physical properties of PAHs

PAHs exist chemically as solids with low volatility at room temperature, high molecular weight and low solubility in water. Physical properties of the sixteen PAHs used in this study are shown in Table 1.1. Relative size of the studied PAHs in comparison to other compounds or among themselves were illustrated by its chemical formula and molecular weight. Molecular weights of PAHs with less than 200 g mol^{-1} are characterized as light PAHs while molecular weights above 200 g mol^{-1} are characterized as heavy PAHs. PAH's affinity are described by the octanol-water coefficient, log K_{oW} while the water solubility of individual PAHs compounds describe whether the involved compound readily transported in the aqueous phase or not. PAHs solubility in water decreases, while correspondingly their boiling and melting point increases, with increasing molecular weight (Albers, 1995).

Table 1.1 Physical and chemical characteristics of studied PAHs (USEPA, 1995; ATSDR, 1995)

PAH	CAS No.	No. of Rings	Chemical Formula	Structural Formula	Molecular Weight	Water Solubility (mg L^{-1} water)	Melting Point (°C)	Boiling Point (°C)
Naphthalene	91-20-3	2	$C_{10}H_{12}$		128	3.17	81	218
Acenaphthylene	208-96-8	3	$C_{12}H_8$		152	3.93	92-93	265-275
Acenaphthene	83-29-9	3	$C_{12}H_{10}$		154	1.93	95	96.2
Fluorene	86-73-7	4	$C_{13}H_{10}$		166	1.68-1.98	116-117	295
Phenanthrene	85-01-8	3	$C_{14}H_{10}$		178	1.20 (at 25°C)	100	340
Anthracene	120-12-7	3	$C_{14}H_{10}$		178	0.0760	218	342

Fluoranthene	206-44-0	4	$C_{16}H_{10}$		202	0.20-0.26	111	375
Pyrene	129-00-00	4	$C_{16}H_{10}$		202	0.077 (at 25°C)	156	393
Benz[a]anthracene	56-55-3	4	$C_{18}H_{12}$		228	0.010	158-159	400
Chrysene	218-01-9	4	$C_{18}H_{12}$		228	$2.8x10^{-3}$	255-256	448
Benzo[b]fluoranthene	205-99-2	5	$C_{20}H_{12}$		252	0.0012	168	No Data
Benzo[k]fluoranthene	207-08-9	5	$C_{20}H_{12}$		252	$7.6x10^{-4}$ (at 25°C)	216	480
Benzo[a]pyrene	50-32-8	5	$C_{20}H_{12}$		252	$2.3x10^{-3}$	179	495

Indeno[1,2,3-cd]pyrene	193-39-5	6	$C_{22}H_{12}$		276	0.062	164	530
Dibenz[a,h]anthracene	53-70-3	5	$C_{22}H_{14}$		278	$5x10^{-4}$	262°C	No Data
Benzo[g,h,i]perylene	191-24-2	6	$C_{22}H_{12}$		276	$2.6x10^{-4}$ (at 25°C)	273	550

1.2.2 Toxicity and carcinogenicity of PAHs

Even though PAHs are being produced naturally in the environment, the amounts produced are less significant than released from human influenced sources. Their presents are studied widely worldwide due to their significant problem to humans and the environment. It been identified that PAHs have toxic effects to the environment and carcinogenic effects to humans and other living organisms.

Sixteen of the PAHs have been listed in Priority Pollutant List by the United State Environmental Protection Agency (USEPA) for their Clean Water Act (USEPA, 2010). The International Agency for Research on Cancer (IARC) has classify 7 of these 16 PAHs namely benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, and indeno[1,2,3- cd]pyrene as carcinogenic since they had carcinogenic effects to the experimental animals and may leave some effects on human as well (IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, 1998; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2002).

Studies on the impact of PAHs exposure towards human are scarce. However, studies on the impact of them towards animals have has been conducted as early as middle of the 20th century and its data has been used as reference for human. PAHs have been reported to defecting body systems through breathing, ingestion or long periods of skin contact with them. In air, PAHs usually bind to the particles or dusts and living organisms can be exposed to it through inhalation. Thyssen *et al*. (1981) and Schulte *et al*. (1993) had recognized benzo[a]pyrene to cause tumors on animals through exposure by breathing while on the other hand, exposure through oral ingestion has been found to cause adverse hematopoietic effects that lead to death on mice (Robinson *et al.,* 1975). It can also cause

gene mutation, difficulties in pregnancies, birth defects, body weight loss, and etc. for the mice (ATSDR, 1995).

Knutzen (1995) has reported the impact of PAHs in waste water towards marine organisms. In his report, PAHs have been said to affect transport across cell membranes causing cell death and react with intermediates to form mutations in DNA, generate a basis for cancer. PAHs have also been said to cause lesions in skin and liver of exposed fish, damaged their reproduction system and induce cataracts.

Among studies related to the impact of PAHs towards human includes a study by Gupta *et al.* (1993) who suggested that benzo[a]pyrene can also impact human through inhalation by destructing human body system. Studies by Man *et al.* (2013) and Purde and Etlin (1980) proposed that exposure of PAHs through dermal can caused cancer. Dermal contact of substances containing PAHs such as tar coal to worker can also cause bad dermatitis and hyperkeratosis (USEPA, 1988).

1.3 Sources and distribution of PAHs in the environments

Polycyclic aromatic hydrocarbons (PAHs) are distributed ubiquitously in the ambient air, soil and water environments, which lead to wide exposure in the general populations (ATSDR, 1995; Li *et al.* 2008). PAHs in the environment can come from natural and anthropogenic sources. Some examples of those sources are shown in Fig. 1.1.

Figure 1.1 Example of anthropogenic (vehicle exhaust & factory emission) and natural sources (forest fire & volcanic eruption) of PAHs

Examples of natural processes that can produce PAHs are incomplete combustion that occurs during plants fire, volcanic activities and diagenetic process (Jenkins *et al.*, 1996; Wilcke *et al.*, 2002; Kim *et al.*, 2003; Zhang *et al.*, 2006). It can also be found naturally in lignite, coal, crude oil and etc. Some of PAHs species such as naphthalene and perylene are said to be produced biologically by woody plants or termites in Amazonian rain forest (Wilcke *et al.*, 2002; Wilcke *et al.*, 2003). PAHs formed biogenically show less variation in chemical structure than those produced industrially (Boitsov *et al.*, 2009).

Anthropogenic activities however dominate as the major producer of PAHs on earth. The sources are from incomplete combustion from vehicles, woods and fossil fuels burning, discharging of waste from industrial, oil spills etc. (Tam *et al.*, 2001; Tham *et al.*,

2008). Some PAHs sources are seasonal (e.g. domestic heating; natural fire events), whilst others are not (e.g. industrial combustion, aluminum and coke production, petroleum refining). Secondary sources of PAHs such as possible volatilisation from soil, water, vegetation or/ and urban surfaces, atmospheric loss or removal processes, such as wet deposition, reactions with OH radicals, scavenging by vegetation are normally influenced by wind speed and direction and mixed boundary layer height (Prevedouros *et al.*, 2004). Sources of PAHs in environment are varied and depending on the location. Determining the sources of PAHs is crucial to assess the level of environmental health in an area. Without reliable information on sources, it is difficult to conceive how a country can accurately assess whether it is handling pollution problems in line with its commitments to international agreements. PAHs usually enter the environment through releases to air from any incomplete combustion activities and settle down into soils or aquatic systems as their final destination (Abou-Arab *et al.*, 2010: Christensen *et al.*, 2007). Fig. 1.2 from Prevedouros *et al.* (2004) explains the fate of PAHs in the environments. It explains that PAHs interact and transferred from one medium to another. Long range atmospheric transport (LRAT) plays an important role in transporting PAHs in atmosphere from one place to another.

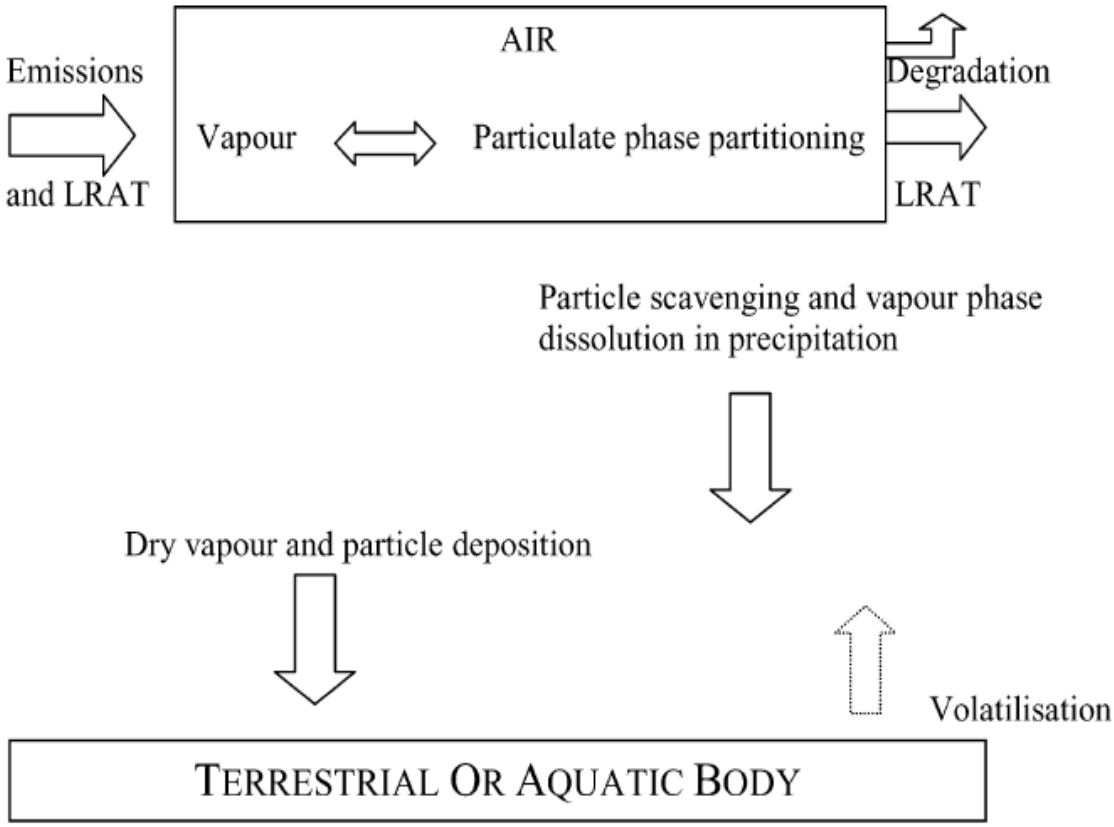


Figure 1.2 Fates of PAHs (Prevedouros *et al.*, 2004)

1.3.1 Aquatic environment

Studies by Santodonato *et al.* (1981) and Jensen (1984) in early 1980's have suggested that most of the PAHs in surface water came from atmospheric deposition. However, major sources of different water body could be different and vary. Other major sources of PAHs into aquatic systems include industrial plants and wastewater treatment plants, urban runoff, oil spills, oil spills, petroleum pressing, etc (Abou-Arab *et al.*, 2010; Duke, 2008; Barrick, 1982; Mackenzie & Hunter, 1979; Giger & Blumer, 1974; Guerin 1978).

In general, PAHs do not easily dissolve in water. They are formed as vapors or attached to the surfaces of small solid particles in air. They can pass through long distances path before they come back to earth in rainfall or particle settling (ATSDR, 1995). PAHs

level in plants and animals living on the land or in water can be so much greater than the level of them in soil or water (Abou- Arab *et al.*, 2010).

Gao *et al.* (2007) explained that under calm conditions in aquatic systems, particles or flocs enriched with PAHs might sink and accumulate in sediments. Microbial and chemical activities might caused some changes when particles settled in the lake's bed and further causing the bound PAHs to be released into the water phase from sediment. PAHs may also enter phytoplankton at the same time they enter the water column. The PAHs in phytoplankton may then bioaccumulate in the food web. Mammals, birds, fishes or any other animals feed the PAH- enriched phytoplankton are subjected to both acute and chronic effects risks of PAHs.

1.3.2 Terrestrial environment

PAHs that are released to the atmosphere can attach to air particulate matter and dusts before settled down into either terrestrial or aquatic systems. While road dusts via road runoff has been well identified as one of the big contributor of PAHs sources in aquatic systems, studies on the sources of PAHs in road dusts are still scarce (Pengchai *et al.*, 2003; Brown *et al.*, 1985; Maltby *et al.*, 1995; Boxall and Maltby, 1995). Pengchai *et al.* (2003) suggested diesel vehicle exhaust, gasoline vehicle exhaust, tire, pavement (asphalt or bitumen), oil spill, etc as the possible sources of PAHs in road dusts. It is believed that among all the possible sources suggested, the main contributor of PAHs in road dusts especially in urban and heavy traffic areas is vehicular exhaust (Lee and Vu, 2010; Takada *et al.*, 1990). It is also believed that PAHs in road dusts may also originate from atmospheric fallout (Takada *et al.*, 1990) and crankcase oil (Zakaria *et al.*, 2002).

On the other hand, most of the PAHs are brought into soil from atmospheric settlement after local and long-range transport, which is supported by the presence of PAHs

in soil of regions remote from any industrial activity (Thomas, 1986). It is believed that soil systems are an important depository for atmospheric PAHs (Gao *et al.*, 2007). Other possible sources of PAHs in soil are disposal from public sewage treatment, irrigation with coke oven effluent, leachate from bituminous coal storage sites, and use of soil compost and fertilizers (Perwak *et al.*, 1982; Santodonato, 1981, Stahl and Davis, 1984; White and Lee, 1980, Jones *et al.*, 1984; Maliszewska- Kordybach, 1993). Owing to their low vapor pressure and high octanol/ air partition coefficients (log K_{OA}), PAHs tend to sorbs strongly onto the soil mass and persevere for a longer period of time (Wilcke, 2000; Gao *et al.*, 2007). Wild and Jones (1995) reported that 90 % of the PAHs are strongly fixed and hence stored in the soils. PAHs accumulated in soil could infect food chains which can further cause direct and indirect contact with human. PAHs in soil might also be transported into the atmosphere and groundwater via few processes namely migration, leaching and evaporation (Gao *et al.*, 2007).

1.4 Degradation of PAHs by bacteria

Bacteria play an important role in the environment. They act as the decomposer in the last stage of food chain. If there were no bacteria, the environment would have been polluted and full of harmful microorganisms.

PAHs own characteristics that make them hard to be utilize of by microbial and encourage their accumulation in the environment. They are resistant to nucleophilic attack, low aqueous solubility and high solid- water distribution ratios. Microbial degradation of PAHs and other hydrophobic substrates is believed to be limited by the amounts dissolved in the water phase (Ogram *et al.*, 1985; Rijnaarts *et al.*, 1990; Volkering *et al.*, 1992; Volkering *et al.*, 1993; Harms and Bosma, 1997; Bosma *et al.*, 1997). The bioavailability of PAHs increases almost logarithmically with decreasing molecular mass, from naphthalene

($C_{10}H_8$) to coronene ($C_{24}H_{12}$). Sometimes, microbes degrade PAHs into metabolites that may transiently or permanently increase toxicity (Belkin *et al.*, 1994; Phillips *et al.*, 2000; Ahtiainen *et al.*, 2002; Donnelly *et al.*, 2005). Frequently, the potentially toxic metabolites are unidentified compounds about which little is known.

According to Mrozik *et al.* (2003), microorganisms that have been found to have capability to transform and degrade PAHs mainly came from the genera of *Pseudomonas* and *Mycobacterium*. Bacterial strains that are able to degrade aromatic hydrocarbons are mainly isolated from soil. Fritsche and Hofrichter (2000) have listed out a few main bacteria that can be found in soils contaminated with aliphatic, aromatic, polycyclic aromatic hydrocarbons and chlorinated compounds. The said bacteria are listed in Table 1.2.

Table 1.2 Common bacteria found in soil contaminated with aliphatic and aromatic hydrocarbons, polycyclic aromatic hydrocarbons, and chlorinated compounds[a]

Gram- Negative Bacteria	Gram- Positive Bacteria
Pseudomonas spp.	*Nocardia* spp.
Acinetobacter spp.	*Mycobacterium* spp.
Alcaligenes sp.	*Corynebacterium* spp.
Flavobacterium/ Cytophaga group	*Arthrobacter* spp.
Xanthomonas spp.	*Bacillus* spp.

[a] Name of the species are not stated as there are changes in some genera and species due to reclassification of bacteria which was done based on phylogenetic markers

Sarma *et al.* (2004) in their studies have isolated bacteria *Leclercia adecarboxylata* from oily- sludge- contaminated soil obtained from the storage pit of oil refinery and applied the bacteria for degradation study of pyrene, catechol, naphthalene, fluorene and fluoranthene. Results of the study shows that within 20 days, 62 % of pyrene had been degraded by *Leclercia adecarboxylata* while 73 %, 53 %, 41 % and 48 % of catechol, naphthalene, fluorene and fluoranthene had been degraded by the same bacteria within the same period of time, respectively. In their studies, 10 g of samples had been inoculated in

200 ml of mineral salt medium (MSM) with 200 mg L^{-1} carbon source (either pyrene, catechol, naphthalene, fluorene or fluoranthene). The culture was incubated at 30 °C on a rotary shaker (200 rpm) for 7 days. After 10 such cycles of enrichment, 1 ml of the culture was diluted 10^8- fold and the diluted culture was plated on MSM agar containing 200 mg L^{-1} of the carbon source and being purified further. Degradation was then performed by inoculating 5 % vol/ vol (10^8 CFU/ ml) bacteria in 200 ml MSM with 200 g L^{-1} sole carbon and the mixture was then incubated at 30 °C in the dark on a rotary shaker (200 rpm) for 20 days. 1ml of the culture was taken out at specific interval for protein analysis and extraction with toluene and chloroform. After being concentrated and solvent exchange, 1ml of the final solution was analyzed with gas chromatography- flame ionization detector (GC- FID).

On the other hand, Abd- Elsalam *et al.* (2009) in their studies had found four degrading bacterial strains namely *Escherichia coli* (EF105548), *Soil bacterium* (EF105549), *Alcaligenes sp*. (EF105546) and *Thiobacter subterraneus* (EF105547) that could degrade 29, 30, 27 and 32 % of anthracene and 43, 48, 34 and 41 % of phenanthrene, respectively. These bacterial strains were isolated from different contaminated soil and water sites in Egypt. The bacterial strains were isolated by incubating 0.1 g of soil samples at 30 ± 2 °C for 24 hour in 50 ml of MSM with phenanthrene or anthracene as the sole carbon source. For enrichment, concentrations of the sole carbon were increased slowly from 10 mg L^{-1} to 500 mg L^{-1}. After the enrichment step, subsamples were taken out and streaked on a solid medium (2 % solidified agar) with 20 mg L^{-1} sole carbon source sprayed on it. The plates were then incubated at 30 °C. The bacteria were then further purified by being picked and streaked on a new minimal agar plates. Degradation studies was performed by real time method and carried out using flourscan apparatus (Labsystem, Finland). 15 µl of the isolated culture were mixed with 278 µl MSM solution in 96 well of ELISA plate (total volume in each well 300 µl). The plate were incubated for 24 hours in

flourscan apparatus which was optimized on template 96- well costar 3596 plate, 30 °C temperature and 200 rpm speed. The measurements were done at every 1 hour for 24 hours.

As there are increasing studies in the degradation capability of bacteria isolated from soil, the curiosity on the capability of bacteria isolated from other component of the earth especially the ones related to soil also increases. Termites are one of the insects that can be found living in soil. They belong to order Isoptera, and are characterized by their colonial behavior. According to Kambhampati and Eggleton (2000), 2650 species of termites have been identified worldwide from 280 genera and 7 families. Among these, Tho (1992) has recorded the existence of 323 species from 52 genera in Indo- Malayan (Oriental) while Thapa (1981) has recorded 104 species from 33 genera in Sabah. Termites are considered ecosystem engineers because of their numerical and ecological significance (Brune and Fredrich, 2000). However, most studies on termite gut microbiota have focused on wood- feeders; analogous studies on other feeding guilds, especially soil- feeders and fungi- cultivators, remain sparse (Kane *et al.*, 2001), owing to their typically remote habitats, delicate nature, and the difficulty of establishing permanent laboratory cultures (Bignell *et al.*, 1986; Rouland *et al.*, 1993).

Species of the genus *Macrotermes* that construct large epigeal nests and extensive underground gallery systems have major effects on soil chemical and physical properties throughout the tropics and subtropics. Most studies on termite- related bacteria focused on their gut bacteria (Slaytor, 1992; Kane *et al.*, 2001; Brune *et al.*, 1995a,b), mainly on the metabolism of monoaromatic compounds such as benzoic acid, cinnamic acid, ferrulic acid and phenylpropanoic acid (Brune *et al.*, 1995a). Studies on bacteria isolated from fungal comb, especially on their capability to degrade aromatic compounds, are still scarce.

1.5 Organic analytical chemistry in environmental research

Reliable and relevant data on the concentrations of pollutants in the environment is necessary for environmental protection policy. The aims of analytical analysis of environmental samples were influenced by the needs of achieving trustworthy measurements at very low concentration and in complex matrices. Two major target areas of interest can be distinguished in the process of development of environmental organic trace analysis. The first area is analytical separation and detection which has been given a big attention in the past and there are many great achievements that have been obtained within a number of decades. The second one is in sample preparation steps which their importance only comes into attention after the highly sensitive analytical systems have become a common standard for environmental analysts (Liśka, 2000). Accuracy of the measurement is usually assessed directly by confirmatory testing or by analyzing certified reference materials.

1.5.1 Methods of extraction

Fritz and Masso (2001) in their study estimated that often, sample preparation takes about 60 % of analysis time while only 33% on sample collection and data handling and 7 % used for instrumental analysis. Nowadays, there is a raising awareness on the importance of fast and efficient methods for sample pretreatment.

There are some difficulties on analyzing PAHs or any other organic pollutant in water by GC- MS. Two common problems occurred in trace analysis of organic pollutants in water by GC- MS are: 1) water samples need to be concentrated as water samples usually are too dilute for direct injection, 2) most GC stationary phase are not compatible with water, thus, direct injection of the water into GC should be avoided. Phase switching of the water has to be done to overcome this problem and one of the techniques is by transferring

the analytes from a large volume of water to a small volume of an organic solvent (Baltussen *et al.*, 1998).

Extraction of semi volatile organic compounds in liquid matrices frequently involves the use of conventional techniques, such as liquid-liquid extraction (LLE) and solid phase extraction (SPE). Compared to SPE, LLE is a time consuming multi step method for which large amounts of solvents are necessary. For that reasons LLE has been largely replaced in past few years by SPE using a variety of sorbents. However unlike LLE, SPE is limited to semi-volatile compounds because the boiling points of the analytes must be substantially above that of the solvents (Eisert and Levsen 1996, Santos and Galceran, 2002, Manoli and Samara, 1999).

A range of methods of extraction and analysis of PAHs in solid medium have been recommended and several studies have been carried out to compare the traditional extraction methods with modern techniques (Luque de Castro and García- Ayuso, 1998; Berset *et al.*, 1999; Budzinski *et al.*, 1999; Lundstedt, 2003). Traditional extraction methods include Soxhlet (USEPA, 1996; Lopez- Avila *et al.*, 1998; Luque de Castro and García-Ayuso, 1998), ultrasonication (Eiceman *et al.*, 1980; Sun *et al.*, 1998; Luque-Garcia and Luque de Castro, 2003) mechanical shaking (Berset *et al.*, 1999) and reflux with methanolic KOH (Wong and Williams, 1980). Modern techniques include soxtec (automated soxhlet) (Lopez- Avila *et al.*, 1993) supercritical fluid extraction (SFE) (Hawthorne *et al.*, 1994; Hawthorne and Grabanski, 2000), microwave- assisted extraction (MAE) (Chee *et al.*, 1996; Camel, 2001), pressurized hot water extraction (PHWE) (Andersson *et al.*, 2002; Juhani *et al.*, 2004) pressurized liquid extraction (PLE) or accelerated solvent extraction (ASE) (Richter *et al.*, 1996; Saim *et al.*, 1998).

Traditional ultrasonic extraction, uses water as agitation energy transportation medium and total recovery can be reached within a relatively short time (usually 45– 60

min) (Lee *et al.*, 2001). In comparison to reflux methods, ultrasonication as an extraction method is an efficient technique for extracting trace organics from solid medium such as soils and sediments. Even though ultrasonic extraction techniques exhibit lower recoveries in some studies (Berset *et al.*, 1999), it has also been proved to generate comparable or even greater quantities (Song *et al.*, 2002) of hydrocarbons than other techniques of extraction.

Sonication can have the advantage of faster extraction times depending on the type of contaminants and matrix. Optimization of the ultrasonic extraction parameters, including type of solvent or solvent composition, extraction time, sample load, and water content shows that repeated extractions were required for more efficient and reproducible extractions (Berset *et al.*, 1999). Ultrasonication techniques usually provide a relatively low cost method, using small volumes of organic solvent without the need of elaborate glassware and instrumentation.

Ultrasonic extraction has proven to be equally or more efficient than Soxhlet extraction. It includes advantages in terms of reproducibility, the applicability of the method to a range of sample sizes, the dramatic reduction in time needed to perform highly efficient extractions, and efficient extraction of polar organic compounds.

Koh (1983) compared Soxhlet method with ultrasonic method for the extraction of solid and found that sonication extraction consume less time, used little bench space, and minimized fire hazard because heating was not required.

A study by Marvin *et al.* (1992) on the reliability of PAHs extraction between Soxhlet and sonication from road dusts and sediments proved that there was no significant difference for recoveries of both techniques. However, between these two methods, ultrasonication method was better in term of time consuming, easiness to operate and capability to extract wide range of sample sizes. Song *et al.* (2002) who compared shaking,

Soxhlet and ultrasonic method for the extraction of 16 listed PAHs by USEPA also found that there was no significant difference in extraction efficiency of all three methods for less polluted samples. However, for highly polluted samples, the efficiency was in the order: shaking < ultrasonic < Soxhlet.

1.5.2 Methods for sample cleanup and fractionation

When the environmental samples contain or are suspected to contain variety of components, sample clean-up and fractionation are required. The purpose of this procedure is to separate groups of compounds so that an aliquot of the sample does not result in a very complicated chromatogram. Furthermore, fractionation steps prior to instrumental analysis are important to separate target compounds and remove potential interferences.

The simplest approach to these steps is passing the sample's extract through a clean-up column to obtain an initial group separation. Wang *et al.* (2011a) in their determination of PAHs in urban surface road dusts of Guangzhou, China used a florisil column to clean-up the extracts as described in EPA Standard Method 3620B (USEPA, 1996) before instrumental analysis.

However, there are many other methods developed by researches to perform these steps. For example, Del Rio *et al.* (1992) in their research for the determination of biomarkers in peat and lignite deposits, applied reflux with isopropanol for 5 hours and left to precipitate in order to remove waxes from resins. Resins, which accounted for approximately 40 % of the whole extracts, were further fractionated using thin layer chromatography (TLC) on silica gel with petroleum ether: diethyler (80:20) as the developing solvent mixture. Different sub-fractions detected by ultraviolet were isolated for further analysis.

A study on the recovery of a few different fractionation methods for the determination of PAHs and aliphatic hydrocarbons has been done by Alzaga *et al.* (2004). In this study, the recoveries of different solid phase extraction (SPE) cartridges (silica, cyanopropyl and silica/ cyanopropyl) were compared with conventional silica- alumina adsorption chromatography. The results show that among these three commercially available cartridges, silica/ cyanopropyl cartridge exhibited the best selectivity for aliphatic and aromatic hydrocarbons separation in two well- resolved fractions, with recoveries of 97 ± 7.2 and 99.7 ± 13.9 %, respectively. The SPE method with silica/ cyanopropyl column was evaluated against the conventional adsorption chromatography and both methods were highly comparable. However, the SPE methodology shows practical advantages in terms of analysis time, consumables, solvent reduction and cost and is particularly suitable for routine applications requiring a high throughput.

Wang *et al.* (2011b) extracted their samples by using Soxhlet extraction method and concentrated the extract to about 1.0 ml. The extract were then passed through an alumina silica packed column (10 cm, 3 % deactivated silica gel, 6 cm, 3 % deactivated alumina, and 1 cm anhydrous sodium sulfate). The silica gel, alumina and anhydrous sodium sulfate were baked at 450 °C for 4 h prior to use. The column was eluted with 50 ml of dichloromethane/ hexane (2:3) at a rate of 2 ml min^{-1} to yield the PAHs fraction. The eluant was concentrated on the rotary evaporator at below 38 °C to approximate 1 ml for instrumental analysis.

1.5.3 Methods of analysis

Numerous analytical techniques have been built up for the determination of PAHs in complex environmental samples. Complexity of the samples and low concentration levels of organic pollutants keep on encouraging the development of research interest and driving

it towards the discovery of new convenient and cost effective methods. A few analytical methods for the determination of PAHs in natural waters are reviewed in Manoli and Samara (1999) which shows the variety of methods available in PAHs and organic compounds analysis.

Gas chromatography (GC) and high performance liquid chromatography (HPLC) coupling with various detectors have been frequently used for identification and quantification of PAHs. Both instruments have their own advantages. HPLC is: (i) well-suited to handle less volatile or non- volatile compounds, (ii) can be used at ambient temperatures and thus acceptable for thermally unstable organic matter, (iii) suitable to be used for wider range of organic compounds especially those with high molecular weight and volatility is not a problem, and (iv) HPLC coupled with fluorescence detector is able to measure some isomers especially PAHs that cannot be quantified easily by gas chromatography- mass spectrometry (GC- MS). On the other hand, advantages of GC includes: (i) simplicity of equipment, (ii) less time consuming, (iii) unparalleled resolution (with capillary column), and (iv) easily interfaced with mass chromatography for compound identification purposes.

According to Berset *et al.* (1999), the determination of PAHs is quite complex especially in the absence of standardized procedures in determining it. A fundamental problem in interpreting analytical results from such investigations is the lack of knowledge on the comparability of data, especially if different analytical methods are used.

1.5.3.1 Gas chromatography- mass spectrometry (GC- MS)

Gas chromatography (GC) is a very popular technique in organic analytical research. The success of this technique in organic matter analysis is due to: (i) its high selectivity and resolution, (ii) good accuracy and precision, (iii) wide dynamic range, and (iv) high

sensitivity. The effectiveness of chromatography and mass spectrometry in chemical analysis is in itself complimentary: GC is a very powerful technique for the separation of organic compounds, meanwhile, MS is a powerful tool for compound identification. However, to utilize this property, only molecules belonging to the same group of substances should be present in the ion source while generating the mass spectrum.

A typical GC- MS system diagram by Masucci and Caldwell (1995) is shown in Fig. 1.3. According to Peters and Moldowan (1993), generally, GC- MS performs six functions in organic separation and analysis. These include:

(i) Separation of compounds through GC; for gas- liquid chromatography, the separation involves the stationary phase and mobile phase in the GC column. The injected molecules will be retained by the stationary phase at the head of the GC column and the process is known as “cold trapping” process. As the temperature of the column rises slowly, the cold- trapped molecules begin to move. The compounds are separated as they are continually retained by the stationary phase and released into the mobile phase depending on their volatility and affinity to each phase.

(ii) Transfer of the separated compounds to the ionizing chamber of the mass spectrometer which is interfaced with the GC.

(iii) Ionization; this function provides all the necessary spectral information required to identify an organic compound whose structure is already known. Electron impact ionization (EI) is the most commonly used ionization technique for this purpose. For structure elucidation of unknown compounds, chemical ionization (CI) and field ionization are frequently used. For EI ionization, each molecule eluting from GC column is bombarded with energetic electrons (normally with 70 eV of ionizing energy) which cause it to form molecular ions, $M^{+\cdot}$. $M^{+\cdot}$ can undergo further fragmentation or rearrangement to form other ions, radical ions or neutral molecules.

(iv) Mass analysis; the function of the mass analyzer is to separate the generated ions from the ion source according to their mass over charge ratio (m/ z) using a quadrupole, magnetic or time- of- flight (Tof) mass spectrometer.

(v) Ion detection by electron multiplier; electron multiplier is used to detect the presence of ion signals emerging from the mass analyzer of a mass spectrometer. During the analysis, the detector measures ions to a mass ratio in range (e.g., m/ z 50 to m/ z 600) at specific time. Each peak, which represents one or more compounds, eluting from the GC produces a distribution of fragment on masses. A mass spectrum shows a plot of relative intensity versus the mass over charge ratio (m/ z) at constant scan number (related time). Fragmentogram (mass chromatogram) is obtained by plotting scan number (related to GC retention time) versus intensity at constant m/z. Magnitude of the total ion current for all mass spectra during a GC- MS analysis when plotted versus scan or retention time on a reconstructed ion chromatography (also call TIC) shows a series of peaks which represent the relative amounts of the eluted compounds.

(vi) Acquisition, processing and display of the data on computer: GC- MS analyses can operate in full- scan mode and selected ion monitoring (SIM) mode. In full- scan mode, each ion current contribution at the detector output can be summed to give a nonspecific chromatographic profile. According to Bruner (1993), this mode can be considered as the best detection mode available for a GC- MS and LC- MS analyses as it can give both quantitative and complete qualitative result. The disadvantage of this mode is the requirement of extensive computer storage and the loss in the acquisition of low intensity ion species, due to the fact that since only a few ions in every mass spectrum have enough intensity to generate a noticeable current. On the other hand, in SIM mode, the abundance of a few selected ions is followed during the chromatographic run. This implies the detection of specific analytes with the consequent loss of information.

However, in complex mixtures, the possibility of selecting only ions of interest may lead to better analytical results. The SIM mode also results in better sensitivity compared to full- scan mode. However, it requires the knowledge of the retention times and fragmentation characteristics of the compounds to be studied.

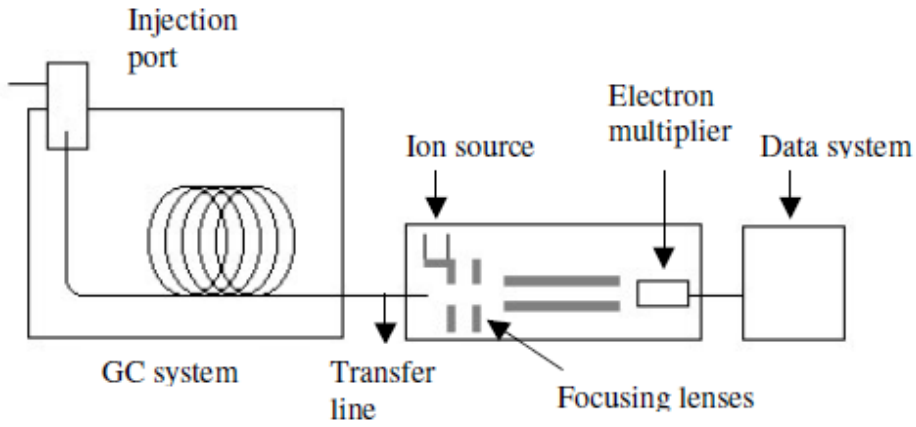


Figure 1.3 Typical GC- MS system diagram (Masucci and Caldwell,1995)

1.5.4 Quality control

Quality control is required to ensure that the analytical procedure functions accurately during practice. It is aimed to guarantee that no unknown changes during analysis influence the analytical result. Precise checking and monitoring procedures must be set so that the researcher can be sure that the whole analytical system always yields tolerable or a fairly good recoveries results.

Validation must always be carried out for newly developed or modified procedures. This takes place either by: (i) analysis of reference materials, or (ii) comparison of the results of the analyses with those of a validated, or (iii) an independent analytical procedure, or (iv) by control samples prepared in the laboratory itself.

1.6 Objectives of the study

The general objectives of this study are:

1) To assess the contamination level and risks of polycyclic aromatic hydrocarbons (PAHs) in two urban recreational lakes, road dust and road side soils of Kuala Lumpur city, Malaysia. In this study, PAHs concentration in a few medium (water, suspended particulate matter (SPM), sediments, road dusts and road side soils) will be determined and a few approaches will be used to identify the sources. Potential risk of the PAHs will be evaluated by using toxicity and cancer risk assessment methods.
2) To evaluate the PAHs level in Kuala Lumpur city as the capital city of Malaysia. Concentration of PAHs determined in this study will be compared with other studies worldwide.
3) To identify the capability of bacteria from fungus comb of soil termite species namely *Macrotermes gilvus* and bacteria from contaminated soils of Kuala Lumpur industrial areas in degrading PAHs. A few bacteria idenfied with this capability will be mixed to form a consortium and their capabilities in degrading PAHs will be evaluated further.

1.7 Thesis outline

Chapter 1: This chapter generally introduces polycyclic aromatic hydrocarbons (PAHs), their source and distribution in the environments. This chapter also introduces degradation capability of bacteria isolated from termites and contaminated soils as well as discussing the analytical method used.

Chapter 2: In this chapter, distributions, sources and toxic potential of PAHs in aquatic environments, specifically in surface water, suspended particulate matter (SPM) and surface sediments of two urban lakes around Kuala Lumpur city are discussed.

Chapter 3: This chapter discusses the distribution, contamination level and risk assessment of PAHs in road side soils and road dusts of industrial, commercial and residential areas located around Kuala Lumpur, Malaysia.

Chapter 4: This chapter discusses the degradation of polycyclic aromatic hydrocarbons (PAHs) by bacterial consortia isolated from contaminated road side soils and fungus comb of soil termite from species *Macrotermes gilvus.*

Chapter 5: This chapter wraps up in general all the chapters discussed earlier and concludes the study.

1.8 References

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Chapter 2

Chapter 2

Identification and Characterization of Polycyclic Aromatic Hydrocarbons in Surface Water, Suspended Particulate Matter and Surface Sediments of Two Urban Recreational Lakes in Kuala Lumpur City, Malaysia.

2.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) from pyrogenic (the incomplete combustion or pyrolysis of organic material) and petrogenic (the direct release of oil or its products) sources are ubiquitous in the global environment and are typically more concentrated near urban centers (Jaward *et al.*, 2012).

Although sources of PAHs in urban areas are numerous, for example, vehicular emissions, industrial emissions, wastewater discharge from domestic and industrial activities, home heating, refuse burning and coal burning at power plants, results from studies in various parts of the world have consistently shown that traffic activities were the major source in urban areas especially in air and soils (Agarwal, 2009; Chung *et al.*, 2007; Hassanien and Abdel- Latif, 2008; Kume *et al.*, 2007; Li *et al.*, 2006; Napier *et al.*, 2008; Okuda *et al.*, 2010). Traffic is also most probably the major source of PAHs in Kuala Lumpur the capital city of Malaysia, which is the most industrialized and economically the fastest growing region in Malaysia. With a population of about 1.7 million and registered motor vehicles of 4.0 million in 2008, vehicular emissions have been identified as the key pollution sources (DOE, 2008; DOS, 2010). Other possible sources of PAHs in Kuala Lumpur are industrial emissions, open burning and the occasional smoke particles from forest fires in Southeast Asia (Omar *et al.*, 2002; 2006). Unlike in temperate countries, home heating and coal combustion are however, not major sources of PAHs in Kuala

Lumpur because temperatures tend to remain warm throughout the year varying from maxima of 31 to 33 $^{\circ}C$ and minima of 22 to 24 $^{\circ}C$. Moreover, there were only 7 coal- fired power plants in Malaysia in 2008 (Othman *et al.*, 2009), all located away from the city. However, there is another significant source of petrogenic PAHs, that is, improper disposal of used crankcase oil and frequent washout of lubricating oil on road surfaces by strong rain (Zakaria *et al.*, 2002). Kuala Lumpur typically receives 2,266 mm (89.2 in) of rain annually. Flooding has been a regular occurrence in Kuala Lumpur whenever there is a heavy downpour, especially in the city center.

Primarily, pyrogenic PAHs are emitted into the atmosphere before other processes take place, and owing to their low aqueous solubility and hydrophobic nature, PAHs tend to associate with particulate material that may then be transported to remote areas far away from the initial source (Natalicio *et al.*, 2011; Ding *et al.*, 2007; Vilanova *et al.*, 2001; Yang *et al.*, 2007; Malik *et al.*, 2011).

PAHs eventually deposit on soils or directly enter the aquatic environment. PAHs accumulated on soil surfaces can be removed by runoff, and finally be deposited in the shallow surface sediments of estuarine, lake and marine environments (Kimbrough and Dickhut, 2006; Christensen and Arora, 2007; Tolosa *et al.*, 2004). Input of PAHs from surface water runoff has increasingly been reported as one of the most frequent causes of surface water and aquatic sediment pollution in urban areas (Boonyatumanond *et al.*, 2006; Motelay- Massei *et al.*, 2006; Murakami *et al.*, 2005; Yang *et al.*, 2010). It is crucial to look into PAHs pollution issues in the aquatic environment as it can harm those ecological systems (Jiang *et al.*, 2007; Feng *et al.*, 2007).

Urban lakes typically have common characteristics such as small size, shallow depth, and a watershed with at least 5 % impervious cover from urban development (Scheuler and Simpson, 2001) exerting a strong influence on such lakes. Urban lakes,

whether natural or man- made are normally used for recreation, water supply, flood control or some other direct human use. However, many urban lakes have been affected by urban expansion and industrial development, and suffer a range of problems due to their shallow water depth and pollution from a variety of anthropogenic sources (Van Metre *et al.*, 2000; Van Metre and Mahler, 2005). Climatic characteristics of this tropical region (i.e., frequent and strong rainstorms) may wash out land based and atmospheric pollutants (e.g. PAHs) to the urban lakes more effectively than in temperate. Urban population growth and industrialization levels inevitably increase the fluxes PAHs from terrestrial and atmospheric sources to urban lakes. Urban lakes tend to have bottom sediments that are enriched in nutrients, trace metals, and PAHs. Van Metre and Mahler (2005) analyzed sediment cores from 10 urban lakes and reservoirs across the United States and found that PAH levels were one to two orders of magnitude higher than during pre- development in the same cores. They concluded that, while PAH levels were only loosely correlated with watershed urbanization, they were closely related to the amount of vehicle traffic in the watershed.

Apart from pollution, many urban lakes have problems due to sedimentation (Charlesworth and Foster, 1991). Hence, urban lakes are subjected to sediment removal in order to regain lost storage capacity of the reservoirs, restore recreational areas, remove nutrient- rich sediments, remove toxic substances, reduce rooted aquatic plant growth, reduce sediment resuspension as well as improve fish habitat. The dredged sediments are disposed by four different methods: 1) flushing the sediments and their water content into nearby rivers or sewers for treatment at a water reclamation works, 2) deposition in landfill sites, 3) transport to a waste treatment plant for dewatering and, 4) incorporation into agricultural soils as slurry (Charlesworth and Foster, 1991). However, incorporating such a slurry into agricultural land may lead to potential contamination of vegetables and food chains by PAHs (Khan *et al.*, 2008; Tao *et al.*, 2004), in turn causing direct or indirect

exposure to man. Moreover, leaching, evaporation and migration are possible PAH sources for atmospheric or groundwater contamination (Cai *et al.*, 2007; Cousins *et al.*, 1999; Ping *et al.*, 2007). Since some PAHs are known to have mutagenic and carcinogenic characteristics (Hui *et al.*, 2009; Ravindra *et al.*, 2008), knowledge of the levels of PAHs in urban lake sediments is needed so that appropriate disposal methods can be chosen to minimize the risk of human exposure and environmental contamination.

In Malaysia, data on the levels of PAHs in the environment are limited. Most studies to date focused mainly on the levels of PAHs in atmospheric particles (Okuda *et al.*, 2002; Omar *et al.*, 2002; 2006), coastal sediments (Elias *et al.*, 2007), soils (Omar *et al.*, 2002; Tahir *et al.*, 2006; Fadzil *et al.*, 2008), tar-balls (Chandru *et al.*, 2008; Zakaria *et al.*, 2002), tobacco smoke (Fadzil and Tahir, 2007), biomass smoke (Tan *et al.*, 2007; Tay *et al.*, 2008) and a remote natural lake (Bakhtiari *et al.*, 2009). Since there are no previous data on PAH concentrations in urban lakes in Malaysia, this study aims to establish whether the lakes are affected by environmental pollution and to determine the background levels for PAHs as baseline data for comparison with future survey data. Studies on PAHs in different compartments (water, suspended particles and sediments) are also important in order to assess the quality of the entire aquatic environment (Maskaoui *et al.*, 2002). Moreover, the results of this study will serve as essential reference information in choosing the most suitable disposal site for dredged sediments.

2.2 Experimental

2.2.1 Sampling sites

Two man- made recreational urban lakes namely Lake Taman Jaya and Lake Perdana (formerly Garden Lake) were chosen for this study (Fig. 2.1a,b). Lake Taman Jaya (lat. 03° 06’ N long. 101° 38’ E) is the first recreational lake in the city of Petaling Jaya located near

one of the busiest roads in Kuala Lumpur, i.e. the Federal Highway on the north, a shopping mall on the west and residential areas on the east and south. Lake Perdana (lat. 03° 08’ N long. 101° 41’ E) is a man- made lake system located surrounded by 92 hectares of parkland on all sides. It is the most popular and the oldest park in Kuala Lumpur. There are a few theme parks built around this lake which include a Butterfly Park, a Deer Park, an Orchid Garden, a Hibiscus Garden and a Bird Park. Lake Taman Jaya has two inlet sources (Stations 1 and 4) which are both from the nearby residential areas with one outlet (Station 3), while Lake Perdana has only one inlet source (Station 4) which is from the surrounding park and one outlet (Station 1). Sediments removal in Lake Taman Jaya was carried out from 2002 to 2004 and dredging for sediment removal in Lake Perdana was initiated in 2007 and completed a year later. The coordinates of sampling stations and their water parameters for both lakes were recorded by using a Global Positioning System (GPS) and a Data Sonde 4a Hydrolab. Data collected are shown in Table 2.1.

(a)

(b)

Figure 2.1 Study areas and sampling locations in (a) Lake Taman Jaya (50 m: 1500 ft) and (b) Lake Perdana (100 m: 300 ft), Kuala Lumpur.

Table 2.1 Coordinates, water parameters, concentrations of suspended particulate matter (SPM) and concentrations of extracted lipids from SPM and surface sediments of Lake Taman Jaya and Lake Perdana, Kuala Lumpur

Location		Coordinates	Depth (m)	Temp ($^{\circ}C$)	Tur (NTU)	pH (units)	SpC (mS cm^{-1})	Sal (‰)	TDS (mmHg)	DO (mg L^{-1})	SPM (mg L^{-1})	TOTAL EXTRACTED LIPIDS(TEL) (mg g^{-1})	
												SPM	Surface Sediments
LAKE TAMAN JAYA	Station 1	N03°06.257' E101°38.967'	2.42	28.32	324.1	8.13	0.171	0.081	0.112	0.43	24.9	8.92	0.75
	Station 2	N03°06.268' E101°38.904'	2.25	28.35	357.0	8.56	0.201	0.091	0.127	1.29	23.5	9.46	3.65
	Station 3	N03°06.357' E101°38.858'	1.50	28.41	333.3	8.31	0.174	0.092	0.121	1.06	24.5	9.07	5.68
	Station 4	N03°06.381' E101°38.874'	1.41	28.33	341.8	8.21	0.221	0.091	0.113	1.31	23.3	9.54	1.94
	Station 5	N03°06.379' E101°38.943'	1.73	28.40	340.2	8.34	0.182	0.702	0.102	0.91	23.8	9.34	4.79
	Station 6	N03°06.327' E101°38.951'	1.29	28.33	320.2	8.24	0.191	0.901	0.115	1.56	32.3	6.88	3.39
LAKE PERDANA	Station 1	N03°08.356' E101°41.120'	1.64	28.95	19.2	8.15	0.094	0.031	0.602	6.53	12.4	8.06	1.09
	Station 2	N03°08.391' E101°41.117'	1.32	28.91	26.1	8.41	0.094	0.042	0.060	6.43	12.8	7.81	0.84
	Station 3	N03°08.511' E101°41.111'	2.21	28.90	20.7	8.48	0.094	0.031	0.061	4.01	14.4	13.91	0.55
	Station 4	N03°08.529' E101°41.105'	1.56	29.22	18.8	8.53	0.095	0.042	0.061	5.91	12.1	8.26	0.95
	Station 5	N03°08.578' E101°41.026'	1.24	28.57	24.1	8.45	0.098	0.041	0.063	3.47	8.1	24.72	0.85

*Temp= Temperature, Tur= Turbidity, SpC= Specific Conductance, Sal= Salinity, TDS= Total Dissolved Solids

2.2.2 Reagent, glassware and apparatus

All solvents used were HPLC grades. Dichloromethane, hexane and methanol were obtained from Merck (Oslo, Norway). Deionised water was obtained from a Milli- Q system (Millipore Co., USA). Filter papers used were glass fiber filters (GF/ F GMF Circles filters, 0.47 mm) from Whatman International (UK). Silica gel (Pharmprep 60 CC, 40- 63 µm) and alumina (70- 230 mesh) was obtained from Merck (Oslo, Norway).

All glassware used in the analytical work were cleaned dried in a few sequential steps which were: a) soaked in 20 % Extran MA 03 Phosphate- free overnight, b) washed with tap water, c) rinsed with distilled water, d) baked in an oven at 200 $^{\circ}C$ overnight, e) wrapped with aluminium foil, and 6) rinsed with methanol and dichloromethane before use.

Silica gel and alumina were cleaned as follows: a) 30 minutes sonication with methanol, b) drying the residual of methanol, c) 30 minutes sonication with n- hexane, and d) further cleaning with dichloromethane- methanol (9:1) using three 15 minutes sonication. Glass wool and anhydrous sodium sulphate were cleaned three times by sonication for 15 minutes period each with methanol and the step was repeated with dichloromethane.

The sieves used were cleaned before used by sonicating for 30 minutes with diluted Extran MA 03 Phosphate- free, washed with tap water and distilled water before air drying.

Freeze- drier used to dry sediments, filter paper with suspended particulate matter (SPM) was a CHRIST, AA- 200 model (New York, USA) while the ultrasonic bath used for sample extraction was WiseClean (San Diego, CA, USA). The rotary- evaporator system used for the concentration steps was of Buchi model (Switzerland).

2.2.3 Standards

2.2.3.1 Internal standards

The internal standard used in this study was a mixture of deuterated internal standards (IS) in EPA 8270 Semivolatile Internal Standard containing dichlorobenzene- d_4, naphthalene- d_8, acenaphthene- d_{10}, phenanthrene- d_{10}, chrysene- d_{12} and perylene- d_{12} each at 2000 mg L^{-1} that were obtained from Supelco (Bellefonte, PA, USA). The internal standards were diluted to a working concentration of 40 mg L^{-1} each.

2.2.3.2 External standards

Standard mixture used was an analytical standard packed in 1 ml ampule that contained 16 compounds specified in EPA method 610, each at 100 to 2000 mg L^{-1} in methanol: methylene chloride. The standard mixture was purchased from Supelco (Bellefonte, PA, USA). It consisted of naphthalene (Naph), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phen), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benz[a]anthracene (BaA), chrysene (Chrys), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenz[a,h]anthracene (DbA), benzo[g,h,i]perylene (BgP) and indeno[1,2,3- cd]pyrene (InP). Standard concentrations of each compound together with the working concentration used were listed in Table 2.2.

The working concentration were prepared by taking out 15 µl of PAHs standard mixture in a clean vial and made up the volume to 200 µl by adding 85 µl methanol: methylene chloride.

Table 2.2 Original concentration and working concentration of every PAHs compounds in the external standard used

Compounds	Concentration (mg L^{-1})	Working Concentration (mg L^{-1})
Naphthalene	989.0	74.18
Acenaphthylene	1978.0	148.35
Acenaphthene	1002.0	75.15
Fluorene	197.1	14.78
Phenanthrene	99.2	7.44
Anthracene	99.8	7.49
Fluoranthene	198.2	14.87
Pyrene	99.2	7.44
Benz[a]anthracene	98.7	7.40
Chrysene	97.6	7.32
Benzo[b]fluoranthene	197.8	14.84
Benzo[k]fluoranthene	99.5	7.46
Benzo[a]pyrene	99.3	7.45
Indeno[1,2,3- cd]pyrene	98.2	7.37
Dibenz[a,h]anthracene	196.4	14.73
Benzo[g,h,i]perylene	196.7	14.75

2.2.4 Sampling and sample preparation

2.2.4.1 Water

Eleven subsurface (0.5 m) water samples from both lakes were collected in August and December 2009. Throughout the sampling process a global positioning system (GPS) was used to locate the sampling positions as shown in Fig. 2.1 (a) and (b) (coordinates of Table 2.1). Water samples (2.5 l) were taken using precleaned amber glass bottles. To collect a sample of the surface layer, the amber glass bottle were held horizontally in the water, half submerged until the bottle were fully filled up with the water. After returning to the laboratory, aliquots of the samples (1 l) were filtered through GF/ F GMF Circular filters (Whatman, 0.47 mm) under vacuum to obtain 1.0 l dissolved samples that were then subjected to extraction step for PAH analysis.

2.2.4.2 Suspended particulate matter (SPM)

Another 1 l the sampled water was filtered through 0.47 mm, GF/ F GMF Circular filters paper (Whatman) to trap the suspended particulate matter (SPM). About 4 to 6 filter papers were used for every sample. The filter papers loaded with the SPM were then kept in a refrigerator at -20 °C. Before extraction, the filter papers were taken out and dried overnight in a freeze- drier and then left in a dessicator to fully remove water.

2.2.4.3 Surface sediment

Surface sediment samples (0- 10 cm) were collected with a mini grab sampler and stored in an aluminum basin (pre- rinsed with methanol, MeOH and dichloromethane, DCM), covered with aluminum foil and kept in an ice chest (temp: ± 4 °C) before been transferred to the laboratory. After returning to the laboratory, the sediments were stored at -20 °C till extraction. The surface sediment samples were also freeze-dried overnight. Freeze-drying is a special form of drying that removes all moisture and tends to have less of an effect (e.g., degradation of compound of interest) on the samples than normal dehydration does. In freeze-drying, sample is frozen and placed in a strong vacuum. The water in the sample then sublimates or turns straight from ice into vapor. Dried sediments were then grinded, sieved through 300 µm mesh to remove unwanted items, homogenized using cone and quartering method and left in a desiccators before extraction.

2.2.5 Extraction

2.2.5.1 Water

Surface water samples were extracted using a solid- phase extraction (SPE) system (Supelco, USA), using the procedure described by Zhou *et al.* (2000). In this method, the Supelco SPE cartridges were first washed with 5 ml ethyl acetate, followed by 5 ml of

MeOH and 2 x 5 ml of deionised water. The filtered surface water samples (1 l) were then passed through the cartridge at a flow rate of 6 ml min^{-1} under vacuum. Following extraction, the cartridge was dried using nitrogen (N_2) and left overnight in a desiccator. The cartridges were then subjected to fractionation step.

2.2.5.2 SPM and surface sediments

SPM and (4 g) surface sediment samples were spiked with deuterated internal standards and extracted three times using ultrasonic agitation for a 15- min period each with 35 ml DCM at a temperature maintained at ≤ 10 °C. The extractions were carried out in a 200 ml conical flask capped with aluminum foil. The extract was then filtered using a glass sintered funnel into a 250 ml flat bottom flask before being subjected to sulfur removal step. Next, the extract was then concentrated on a rotary evaporator to a volume of approximately 2 ml, which was then adjusted to 2 ml exactly by addition of DCM. A 1 ml aliquot of the extracts was transferred to a pre- weighted vial and left to dry to measure the Total Extracted Lipid (TEL) of the samples while the other 1 ml (for SPM) and 500 µl (for surface sediments) of the concentrated extracts were subjected to the fractionation step.

2.2.6 Sulfur removal

Extracted SPM and surface sediments samples were subjected to sulfur removal step before undergoing the fractionation step. In this step, activated copper wires were added in the extracts and the samples were shaken overnight (~8 hours). Elemental sulfur need to be removed as it could interfere in GC- MS reading/ analysis. Blackening of the copper indicates reaction with the elemental sulfur has occurred which indicates the requirement of this step to be repeated until the color of the copper remains unchanged. Each extract was then filtered through glass wool to remove the copper.

Copper wire used in this analysis was 99.999 % trace metal assay and purchased from Sigma- Aldrich (St. Louis, MO, USA). It was first cut in small pieces and activated by washing it with concentrated (37 %) hydrochloric acid in an Erlenmeyer flask until the color of the copper changed into bright color. The copper cubes were then rinsed with distilled water to neutralize the pH, followed by MeOH and DCM 7 times each.

2.2.7 Fractionation

2.2.7.1 Water

Separation for the water samples was carried out by eluting the dried cartridge with 8 ml of n- hexane: dichloromethane (1:1) and the eluate was then reduced in volume by N_2 blow and adjusted to be exactly 20 µl.

2.2.7.2 SPM and surface sediments

The extracts were fractionated by using silica- alumina column chromatography. A glass column (20 cm x 1.0 cm i.d.) were used with 5 g of partially deactivated silica gel (Merck, Pharmprep 60 CC, 40- 63 µm) and 10 g of neutral alumina (on top) (Merck, 70-230 mesh). Both silica gel and alumina had been activated at 200 °C for 4 hour and deactivated with 5 % deionised water. The narrow end of the column was plugged with pre- cleaned glass wool while the top of the column were capped with 1 g of anhydrous sodium sulfate. Sodium sulfate was used to form a protective surface layer to the column as well as a water trapper.

After placing the extracts on the top of the column, the column was first eluted with 14 ml of hexane and the eluate discarded. This elution was expected to take out the aliphatic hydrocarbons from the extract. The second fraction which was rich with PAHs were then obtained by eluting the extracts with 30 ml of 10 % DCM in hexane followed by

20 ml of 50 % DCM in hexane. This fraction was concentrated to 200 µl by using rotary evaporator at 40 °C under reduced pressure, and transferred into a 2 ml vial with Teflon-lined cap for gas chromatography- mass spectra (GC- MS) analysis.

2.2.7.3 Optimization of fractionation procedure

Alumina and silica gel were used as absorbents in the fractionation step. Organic chemists usually used these absorbents for column chromatography studies. Neutral alumina with 70- 230 mesh and silica gel with 60 CC, 40- 63 µm were chosen to give better separation and satisfactory flow rate.

Neutral alumina was selected instead of other basic alumina so that the original forms of the compounds of interest can be maintained. Basic alumina may caused hydrolysis of ester while the acidic alumina may lead to dehydration of alcohols, particularly tertiary alcohols and might also cause an isomerisation of carbon- carbon double bonds. Neutral alumina selected in this study is the most active grade which has a strong capability to retain polar compounds.

Partially deactivation of pre-activated silica gel and neutral alumina with 5 % of deionised water were done to regulate the activity of the absorbents as most of the separations work well with at least 3- 5 % moisture. Jeffery *et al.* (1978) reported that addition of water to the absorbents would lead to the formation of substantial film of surface so that a column prepared from such material may be used to affect separations by partition rather than adsorption.

2.2.8 Instrumental analysis

The determination of PAHs was performed by gas chromatography- mass spectrometry (GC- MS) using a QP2010 Plus instrument (Shimadzu, Japan) equipped with RTX- 5MS (Crossbond 5 % diphenyl/ 95 % dimethylpolysiloxane) column (30 m x 0.25 mm i.d., 0.25

µm film thickness). The carrier gas used was purified helium (99.999 % purity) with flow rate at 1.20 ml min^{-1}. The chromatographic conditions of the GC are presented in Table 2.3. The data for quantitative analysis was acquired from electron impact (EI) mode (70 eV) and the data were acquired under selected ion monitoring (SIM) mode.

Table 2.3 Chromatographic conditions

Program	**Conditions**
Oven temperature program	Initial temperature at 70 °C, held for 2 min; increased at a rate of 30 °C min^{-1} to 150 °C, and then increased at a rate of 4 °C min^{-1} to 310 °C before held isothermal for 10 min.
Gas flow rates	1.20 ml min^{-1}
Temperature of injection port	300 °C
Injection mode	Splitless (1 min), 1 µl; hot needle technique
Column inlet pressure	10.4 psi
Average velocity	40 cm s^{-1}
Solvent delay	4 min

Briefly, the alumina- silica gel fractionation prior to GC- MS analysis should reduce the interference from organic compounds complex mixture of the samples. However, the alumina- silica gel fractionation column would not separate everything especially compounds with similar polarities, in this case, analysis by GC- MS by using SIM mode would solve the problem. The usage of SIM mode can minimize the interference from other compounds and the compounds of interest could be identified and quantified easily. Operation of a GC-MS in SIM mode allows for detection of specific analytes with increased sensitivity relative to full scan mode. In SIM mode the MS gathers data for masses of interest rather than looking for all masses over a wide range. Because the instrument is set to look for only masses of interest it can be specific for a particular analyte of interest. Typically two to four ions are monitored per compound and the ratios of those ions will be unique to the analyte of interest. In order to increase sensitivity, the mass scan rate and dwell times (the time spent looking at each mass) are adjusted. When properly

setup and calibrated, GC-MS with SIM mode can increase sensitivity by a factor of 10 to 100 times that of GC/MS-Full Scan. Because unwanted ions are being filtered, the selectivity is greatly enhanced providing an additional tool to eliminate difficult matrix interferences.

Identification of PAHs was based on the selected ions and the comparison of retention time between samples and the standard solution containing individual PAHs. When the fractionated extract injected into the GC, it swept onto a separation column by helium (inert carrier gas used). The analytes in the mixture are carried through the column by the carrier gas where they are separated from one another by their interaction between the coating (stationary phase) on the inside wall of the column and the carrier gas. Each analyte interacts with the stationary phase at different rates. Those that react very little move through the column quickly and will exit into the mass spectrometer before those analytes having longer interaction and retention times.

When the individual analytes exit the GC column they enter the ionization area (ion source) of the MS. Here they are bombarded with electrons which form ionized fragments of the analyte. These ionized fragments are then accelerated into the quadrapole via a series of lenses and separated based on their mass to charge ratio. This separation is accomplished by applying alternating RF (radio frequency) and DC (direct current) voltage to diagonally opposite ends of the quadrapole, which in turn allows a specific mass fragment to pass through the quadrapole filter. From here the fragments enter the mass detector (electron multiplier) and are recorded. The MS computer graphs a mass spectrum scan showing the abundance of each ionized mass fragment.Quantitative determination of individual PAHs was performed by using most abundant ion. Table 2.4 shows the group list of PAHs, the quantifications and confirmation ions for each PAH identified and quantified while Fig. 2.2 shows the example of separation by SIM mode in chromatogram.

Table 2.4 List of analyte PAH groups, their quantification ions and the confirmation ions for SIM GC- MS analyses

Group	Compound	Range of time (min)	Quantification ion	Confirmation ions
1	Naphthalene	4.00- 7.00	128	102, 125, 127, 129
	Naphthalene- d_8		136	136
2	Acenaphthylene	7.00- 9.50	152	125, 151, 153
	Acenaphthene		154	125, 152, 153
	Acenaphthene- d_{10}		164	164
3	Fluorene	9.50- 12.0	166	139, 163, 165
4	Phenanthrene	12.0- 16.0	178	152, 176, 179
	Anthracene		178	152, 176, 179
	Phenanthrene- d_{10}		188	188
5	Fluoranthene	16.0- 25.0	202	200, 201, 203
	Pyrene		202	200, 201, 203
6	Chrysene	25.0- 30.0	228	200, 227, 229
	Benz[a]anthracene		228	202, 226, 229
	Chrysene- d_{12}		240	240
7	Benzo[b]fluoranthene	30.0- 37.0	252	125, 126, 250, 253
	Benzo[k]fluoranthene		252	125, 126, 250, 253
	Benzo[a]pyrene		252	125, 126, 250, 253
	Perylene- d_{12}		264	264
8	Indeno[1,2,3- cd]pyrene	37.0- 45.0	276	138, 274, 277
	Dibenz[a,h]anthracene		278	139, 276, 279
	Benzo[g,h,i]perylene		276	138, 274, 277

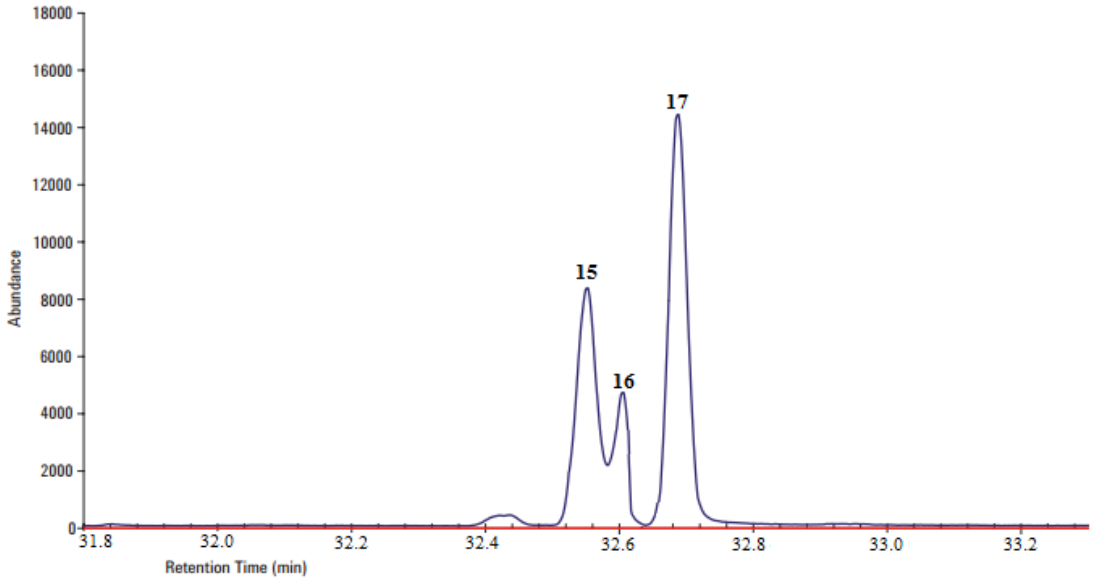

Figure 2.2 Seperation of benzo[b]fluoranthene (15), benzo[k]fluoranthene (16) and benzo[a]pyrene (17), (m/z 252)

The concentrations of PAHs were calculated by using equation as per below:

$$\textbf{Conc (µg kg}^{-1}\textbf{ dw) = Amt / W} \qquad \text{Equation 2.1}$$

Where,

$$\textbf{Amt= ((RF}_{\textbf{avg}}\textbf{ x Area x V}_{\textbf{binj}}\textbf{) / (V}_{\textbf{inj}}\textbf{ x V}_{\textbf{ext}}\textbf{) / (V}_{\textbf{frc}}\textbf{ – A}_{\textbf{contm}}\textbf{)) / (R/ 100)} \qquad \text{Equation 2.2}$$

Where,

Amt is the amount of compound corrected to blank and recovery.

RF_{avg} is the average response factor ($RF_{avg} = Conc_{ext} \times V_{inj} / Area_{ext}$)

Where,

$Conc_{ext}$ is the concentration of the external standard,

V_{inj} is the injection volume,

$Area_{ext}$ is the area of the external standard in the fragmentogram.

Areas in the formula are referring to the peak area of the compound in the fragmentogram,

V_{binj} is the total volume before injection into GC,

V_{ext} is the volume of the extract,

V_{frc} is the volume of the extract used for fractionation,

A_{contm} is the amount of contamination ($A_{contm} = Conc_{contm} \times V_{ext}$)

Where,

$Conc_{contm}$ is the contaminant concentration,

V_{ext} is the total volume of the extract of the sample to be corrected,

R is the recovery

W is the weight of either SPM or surface sediments.

2.2.8.1 Optimization of instrumental analysis procedure

The optimization of instrumental analysis procedure in this study was done based on theoretical aspects. In this study, a non- polar column namely RTX- 5MS (Crossbond 5 % diphenyl/ 95 % dimethylpolysiloxane) was used since the compound of interest, i.e PAHs is a non- polar, hydrophobic compounds and do not ionize.

The carrier gas linear velocity or flow rate directly influences retention time and efficiency of the analysis. In this study, the carrier gas chosen is helium as it is safer than hydrogen gas which doses explosive risk even though hydrogen gas has better resolving capability and has less viscosity. Helium gas was also chosen because it has greater resolving ability, inertness, and consumed less time for analysis compared to nitrogen gas.

In this study, 10 psi initial pressure and 1.2 ml min^{-1} initial flow rate were applied and adjusted based on the average velocity of the mobile phase. Based on van Deemter curve, the average velocity of 40 cm s^{-1} was suitable for best separation and faster analysis.

As the samples analyzed were in a trace amount, the splitless injection mode was chosen as the method of injection. To ensure most of the samples enter column properly, the splitless time of the injection was adjusted to be 1 minute. As the inner volume of the

injection port is 0.4 ml, the sample was expected to enter the GC column in 20 seconds with the carrier gas flowing at 1.2 ml min^{-1}.

The temperature program applied was as per described by Zakaria *et al.* (2001). The initial temperature of the oven was hold at 70 °C for 2 minutes, then programmed at 30 °C min^{-1} to 150 °C, 4 °C min^{-1} to 310 °C and held for 10 min.

Electron ionization mode was selected for the mass spectrometric conditions. Each molecule eluted from the GC column was bombarded with electron having 70 eV. As most of the molecules efficiently ionized in the range of 50 eV to 90 eV, 70 eV was decided to be the most suitable based on the empirical observation.

2.2.9 Quality assurance

In this study, all analytical data were subjected to strict quality control. The procedural blanks, spiked blanks, recoveries, GC reproducibility, sample duplicates were routinely analyzed with the samples and all data were corrected for blank.

2.2.9.1 Reproducibility

Reproducibility is the ability of an entire experiment or study to be reproduced, either by the researcher or by someone else working independently. It is one of the main principles of thescientific method. In this study, reproducibility of the gas chromatographic analysis was examined by three injections of PAHs standards and the internal standards. The results are shown in Table 2.5. The reproducibility of the chromatographic procedure was assessed by performing three injections on different days. With R.S.D. less than 12%, the reproducibility of the study was considered good. The value were probably varies due to differences in operators conditions in every injection days, laboratory conditions (e.g., humidity, temperature, etc.), apparatus conditions as well as time between tests.

Table 2.5 Reproducibility of GC- MS instrument expressed as percentage (%) Standard Deviation

Compounds	R.S.D. (%), n= 3
Naphthalene	0.92
Acenaphthylene	1.61
Acenaphthene	2.01
Fluorene	1.01
Phenanthrene	1.30
Anthracene	2.25
Fluoranthene	1.68
Pyrene	3.23
Benz[a]anthracene	1.59
Chrysene	1.16
Benzo[b]fluoranthene	1.31
Benzo[k]fluoranthene	1.16
Benzo[a]pyrene	0.74
Indeno[1,2,3- cd]pyrene	5.01
Dibenz[a,h]anthracene	11.4
Benzo[g,h,i]perylene	4.63
Naphthalene- d_8	1.40
Acenaphtene- d_{10}	1.65
Phenanthrene- d_{10}	2.92
Chrysene- d_{12}	4.71
Perylene- d_{12}	0.68

2.2.9.2 Procedural blanks

Procedural blanks for the extraction and fractionation steps were carried out using only dichloromethane following the procedures described in Sections 2.2.5 and 2.2.7 respectively. It was followed by GC- MS analysis. The experiment was repeated three times.

Solvent blanks experiments were also carried out from time to time to monitor the background of the GC- MS. In this procedure, 450 ml DCM and 50 ml of hexane were mixed and concentrated to 100 µl. Peaks that appeared in the chromatogram of GC- MS for this step were quantified and the amounts were subtracted from the real samples so that the actual concentration of the compounds of interest can be obtained. While subtraction of the solvent blank run is particularly important for estimation of total PAH content, it will

generally make peak area integration easier and more accurate for all data processing. An example is given below for PAH in surface sediment (Fig. 2.3). Integration of the fifteen standard peaks of interest (peak labels 1 – 15) is simpler and more accurate after subtraction of the blank run.

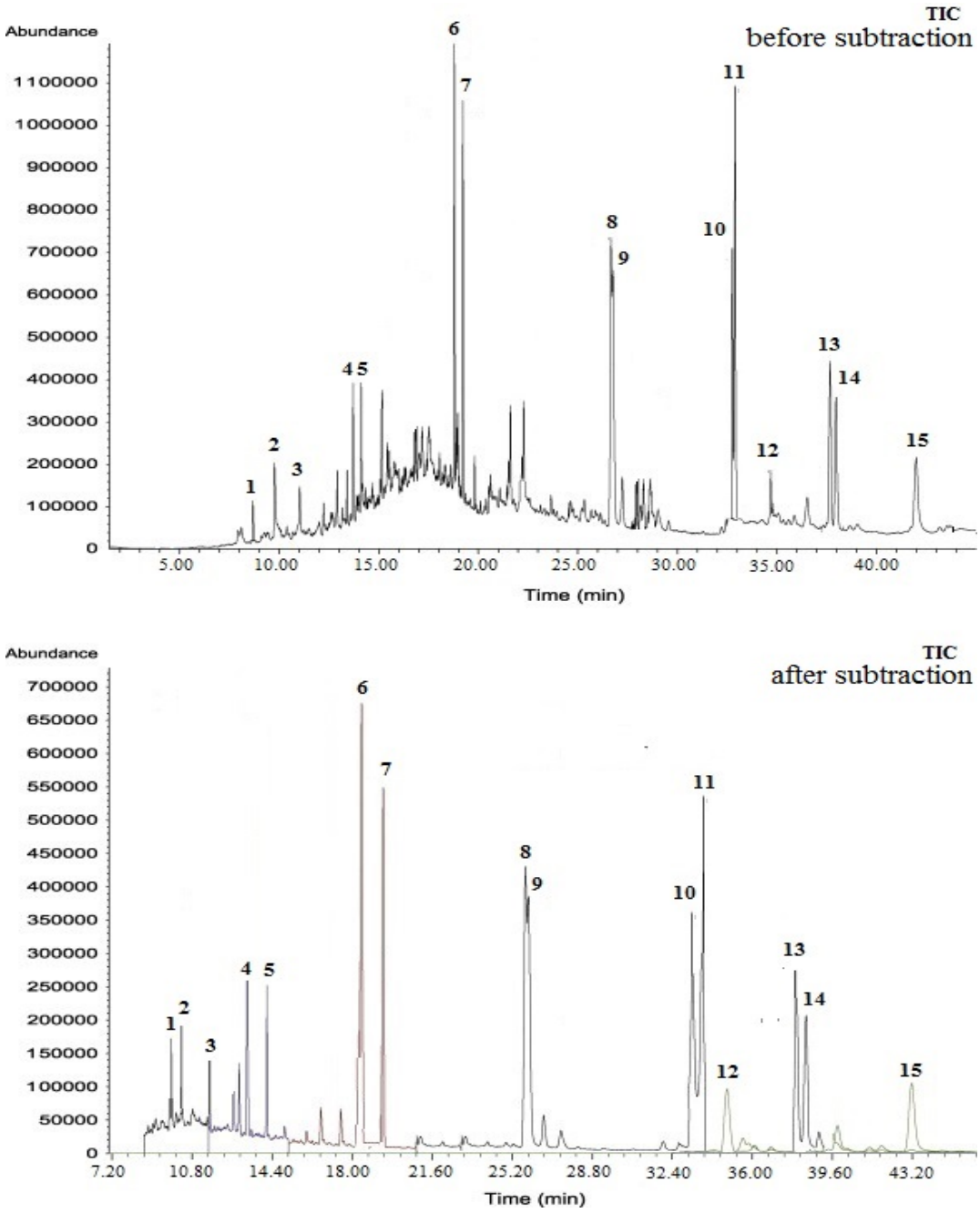

Figure 2.3 Chromatogram of PAHs in surface sediment before subtraction (upper trace) and after subtraction (lower trace) of the solvent blank chromatogram

2.2.9.3 Detection limits

2.2.9.3.1 Instrument detection limit (IDL)

Even when a blank is analyzed, GC- MS will produce a signal which is known as the noise (Fig. 2.4). Most analytical instruments are like this. The IDL is the analyte concentration that is required to produce a signal greater than three times the standard deviation of the noise level. The GC- MS IDL in this study was determined by injecting the blank for three times and the signals of the instrument were recorded. The IDL were then measured by multiplying the standard deviation of the instrument responses with three. Table 2.6 shows the detection limit of the GC- MS to the signals of compounds of interest in mg L^{-1}.

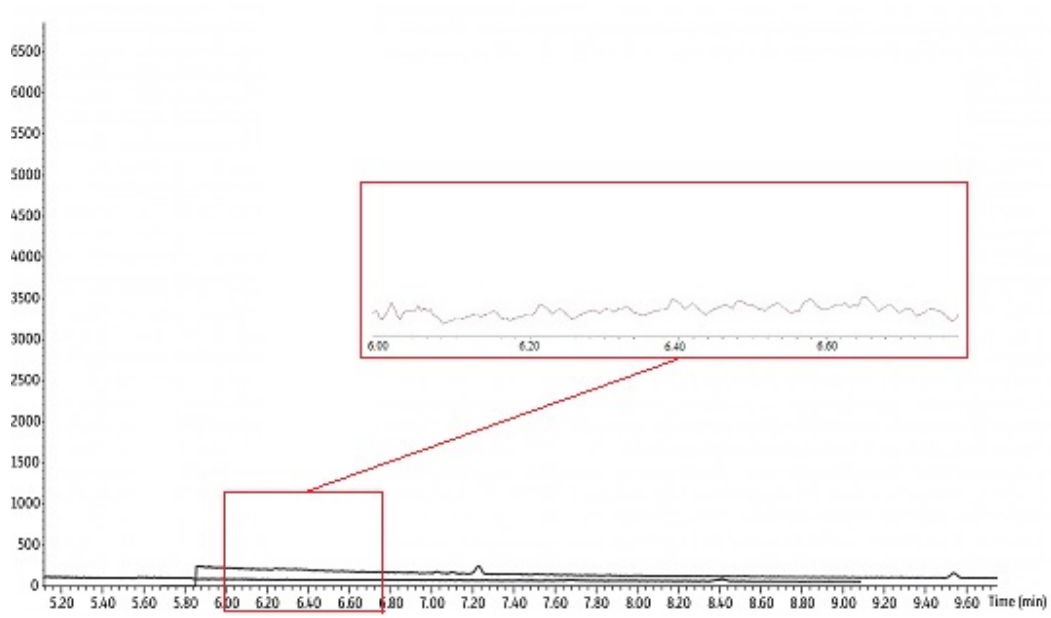

Figure 2.4 Chromatogram of GG-MS signal (noise) when blank is analyzed

$$\textbf{Instrument Detection Limit (IDL)} = \textbf{Standard deviation (S.D.) of the instrument responses} \times 3 \qquad \text{Equation 2.3}$$

Table 2.6 GC- MS Instrument detection limit (IDL) for this PAHs study

Compounds	**IDL (**mg L^{-1}**)**
Naphthalene	1.06
Acenaphthylene	2.67
Acenaphthene	2.55
Fluorene	2.43
Phenanthrene	1.73
Anthracene	3.47
Fluoranthene	2.00
Pyrene	2.17
Benz[a]anthracene	1.91
Chrysene	3.21
Benzo[b]fluoranthene	5.80
Benzo[k]fluoranthene	4.83
Benzo[a]pyrene	2.49
Indeno[1,2,3- cd]pyrene	13.41
Dibenz[a,h]anthracene	18.95
Benzo[g,h,i]perylene	8.03
Naphthalene- d_8	0.96
Acenaphtene- d_{10}	1.32
Phenanthrene- d_{10}	1.01
Chrysene- d_{12}	2.52
Perylene- d_{12}	1.96

2.2.9.3.2 Method detection limit (MDL)

Method detection limit can be define as the smallest amount of the analyte of interest that can be detected and reported with 98 % confident that the concentration is greater than zero. The MDL was measured by adding 50 µl of standard solutions to the blank and run it through step 2.2.5 and 2.2.7 (Table 2.7). These procedures were repeated for 3 times and MDL calculated according to the following equation:

$$\textbf{Method Detection Limit (MDL)} = \textbf{Standard deviation (S.D.) of the results} \times \textbf{3.14}$$

Equation 2.4

Table 2.7 GC- MS Method detection limit (MDL) for this PAHs study

Compounds	MDL		
	Surface Water (ng L^{-1})	SPM (mg kg^{-1})	Surface Sediments (mg kg^{-1})
Naphthalene	0.45	0.55	0.52
Acenaphthylene	0.32	0.29	0.28
Acenaphthene	0.25	0.31	0.28
Fluorene	0.30	0.25	0.39
Phenanthrene	0.27	0.33	0.39
Anthracene	0.28	0.27	0.33
Fluoranthene	0.28	0.21	0.29
Pyrene	0.23	0.29	0.26
Benz[a]anthracene	0.25	0.28	0.23
Chrysene	0.33	0.30	0.30
Benzo[b]fluoranthene	0.25	0.23	0.21
Benzo[k]fluoranthene	0.18	0.22	0.12
Benzo[a]pyrene	0.16	0.13	0.09
Indeno[1,2,3- cd]pyrene	0.31	0.28	0.25
Dibenz[a,h]anthracene	0.18	0.13	0.09
Benzo[g,h,i]perylene	0.22	0.21	0.21
Naphthalene- d_8	0.52	0.43	0.50
Acenaphtene- d_{10}	0.42	0.23	0.63
Phenanthrene- d_{10}	0.34	0.41	0.58
Chrysene- d_{12}	0.28	0.21	0.25
Perylene- d_{12}	0.27	0.33	0.85

2.2.9.4 Recovery Studies

2.2.9.4.1 Recovery of the fractionation step

Recovery study for SPM and surface sediments' fractionation step was done. The recovery for alumina- silica gel columns were studied by applying 500 µl of PAHs working standard as listed in Table 2.2 to the top of a glass column (20 cm x 1.0 m i.d.) which was with 5 g of partially deactivated silica gel (Merck, Pharmprep 60 CC, 40- 63 µm) and 10 g of partially deactivated neutral alumina (on top) (Merck, 70- 230 mesh). Elution factors used were similar to those described in Section 2.2.7.2. Table 2.8 shows the result of the recovery study. The recovery was satisfactory for most of the compounds except for the

lower molecular weight compounds which might be due to their high tendency to be volatized.

Table 2.8 Mean ± Standard Deviation for fractionation step (n= 3) in percentage (%)

Compounds	% Recovery ($\bar{x}$ ± R.S.D, n= 3)
Naphthalene	74.3 ± 4.5
Acenaphthylene	78.1 ± 4.1
Acenaphthene	102.5 ± 7.6
Fluorene	95.1 ± 10.0
Phenanthrene	104.3 ± 3.0
Anthracene	97.7 ± 5.6
Fluoranthene	99.1 ± 9.8
Pyrene	103.9 ± 3.2
Benz[a]anthracene	81.9 ± 8.1
Chrysene	96.7 ± 1.9
Benzo[b]fluoranthene	92.1 ± 5.2
Benzo[k]fluoranthene	88.9 ± 2.3
Benzo[a]pyrene	90.6 ± 3.3
Indeno[1,2,3- cd]pyrene	97.9 ± 5.4
Dibenz[a,h]anthracene	89.9 ± 1.7
Benzo[g,h,i]perylene	95.7 ± 2.5
Naphthalene- d_8	82.3 ± 4.5
Acenaphtene- d_{10}	93.6 ± 7.2
Phenanthrene- d_{10}	102.4 ± 9.8
Chrysene- d_{12}	95.8 ± 8.1
Perylene- d_{12}	110.2 ± 5.9

2.2.9.4.2 Multi- step recovery

Multi step recoveries were studied for all samples by using blank samples. Deionised water was used as a blank for surface water recovery study while clean filter papers were used for SPM study. As for surface sediments, their blank samples were prepared by extracting the samples with DCM: MeOH (3: 1) repeatedly and the solvent extracts were injected into GC- MS. The samples were assumed to be clean when there was no contaminant peak appearing in the chromatogram recorded.

All blank samples were spiked with PAHs standards solutions and surrogate solutions before undergoing the procedures described in sections 2.2.5- 2.2.8 and the results of the recovery are shown in Table 2.9.

Surrogates are organic compounds that are similar in chemical composition to the analytes of interest and spiked into environmental and batch QC samples prior to sample preparation and analysis. Surrogate recoveries for environmental samples are used to evaluate matrix interference on a sample- specific basis. However, in order for this approach to be viable, the surrogates must behave in the same manner as the corresponding target analytes that are native to the matrices of interest (e.g., must partition between various phases in the same manner as the native target analytes). Unfortunately, in practice, this equivalency is typically difficult to demonstrate and is often more assumed than empirically derived. The most representative surrogate will typically be an isotopically- modified version of the target analyte. Therefore, when evaluating surrogate results, the representativeness of the surrogates should always be taken into account. In this study, the surrogate compounds used were acenaphthene- d_{10}, phenanthrene- d_{10}, chrysene- d_{12} and perylene- d_{12}.

Table 2.9 The results of multi- step recoveries including the surrogates' recoveries in percentage for surface water, SPM and surface sediments

Compounds	% Recovery ($\bar{x}$ ± R.S.D, n= 3)		
	Surface Water	SPM	Surface Sediments
Naphthalene	59.3 ± 27.4	28.8 ± 22.1	27.5 ± 12.3
Acenaphthylene	58.1 ± 15.1	57.8 ± 11.4	54.7 ± 8.3
Acenaphthene	60.3 ± 10.9	53.7 ± 12.7	58.7 ± 18.0
Fluorene	55.8 ± 7.6	26.4 ± 13.2	26.3 ± 17.0
Phenanthrene	64.6 ± 21.0	72.9 ± 13.1	71.9 ± 13.5
Anthracene	73.7 ± 12.5	70.5 ± 6.0	65.6 ± 5.3
Fluoranthene	61.4 ± 25.4	69.3 ± 5.0	54.5 ± 7.1
Pyrene	69.2 ± 15.7	75.7 ± 11.4	74.9 ± 11.4
Benz[a]anthracene	70.6 ± 15.2	87.6 ± 5.3	82.7 ± 14.1
Chrysene	58.5 ± 21.6	63.9 ± 25.0	70.1 ± 6.04
Benzo[b]fluoranthene	67.5 ± 17.9	79.5 ± 22.2	75.5 ± 12.2
Benzo[k]fluoranthene	71.1 ± 19.4	89.3 ± 11.0	88.9 ± 22.2
Benzo[a]pyrene	77.2 ± 16.1	83.6 ± 21.1	75.8 ± 21.9
Indeno[1,2,3- cd]pyrene	55.1 ± 23.9	55.1 ± 17.9	61.9 ± 14.3
Dibenz[a,h]anthracene	79.7 ± 19.3	68.1 ± 13.2	74.7 ± 12.3
Benzo[g,h,i]perylene	117.0 ± 21.1	76.3 ± 13.9	82.7 ± 24.1
Acenaphtene- d_{10}	55.1 ± 19.0	57.7 ± 22.5	61.6 ± 21.2
Phenanthrene- d_{10}	63.5 ± 20.2	55.3 ± 26.1	63.9 ± 25.8
Chrysene- d_{12}	59.4 ± 24.1	62.3 ± 23.9	66.2 ± 21.7
Perylene- d_{12}	59.7 ± 21.9	68.4 ± 25.1	102.1 ± 27.0

Multi- step recoveries were confirmed by running a certified reference materials: PAH's in harbour sediments (Certified Reference Material (CRM) No 535, Sample Identification No.: 216) from the European Commission, Community Bureau of Reference through the same steps. CRMs can be used to assess the accuracy and precision of methods on long- term basis, as described by ISO- Guide (ISO Guide 33, 2000). The results obtained were in good agreement with the certified values and shown in Table 2.10.

Table 2.10 The results of multi- step recoveries Certified Reference Materials (CRM) No 535: PAH's in harbour sediments

Compound	Freshwater Harbour Sediment (CRM- 535)		
	Certified Conc. ($\mu g\ g^{-1}$)	Found Conc. ($\mu g\ g^{-1}$)	% Error
Pyrene	2.52 ± 0.18	2.56 ± 0.61	1.59
Benz[a]anthracene	1.54 ± 0.10	1.43 ± 0.72	7.14
Benzo[a]pyrene	1.16 ± 0.10	1.32 ± 0.76	13.82
Benzo(e)pyrene	1.86 ± 0.13	1.75 ± 0.12	5.91
Benzo[b]fluoranthene	2.29 ± 0.15	2.01 ± 0.32	12.23
Benzo[k]fluoranthene	1.09 ± 0.15	1.15 ± 0.44	5.50
Indeno[1,2,3- cd]pyrene	1.56 ± 0.14	1.61 ± 0.11	2.56

The recoveries of lower molecular weight PAHs were found to be low in recovery study by using spiked standard solution (Table 2.9) in comparison to the study by using CRM (Table 2.10). This difference was expected to happen due to analyte in spiked solution often cannot be integrated in the sample in the same manner as the original analyte, and then treatments such as extraction may not necessarily reflect the behaviour of real samples.

2.3 Results and discussion

Among the six samples of surface water and surface sediments collected in Lake Taman Jaya, two were from the inlets, one from the outlet and the other three random around the lake (Fig. 2.1a). The five samples of surface water and surface sediments in Lake Perdana were collected one each at the inlet and outlet, and the other three randomly (Fig. 2.1b). The levels of PAHs in water, SPM and sediments will be discussed here to assess the current contamination status by PAHs in the two lakes.

2.3.1 PAHs in surface water

The yields for PAHs in surface water of both lakes are given in Table 2.11 (a) and (b). The total concentration in water for Lake Taman Jaya were corrected for the extraction efficiency and ranged from 563 (Station 5) to 1185 ng L^{-1} (Station 1), with a mean of 734

ng L^{-1} (Table 2.11a). Station 1, located in front of one inlet, had the highest concentration that (wash- out) water from rain drainage flows into the lake from surrounding residential areas and contributes significantly towards the level of contaminants including PAHs in the lake. Furthermore, the location of the lake adjacent to one of the busiest highways in Kuala Lumpur (Federal Highway) represents another input of PAHs from vehicle exhaust. This is supported by the work of Omar *et al.* (2002) which shows that PAHs in atmosphere of Kuala Lumpur are derived mainly from vehicular exhausts. Many vehicles pass through this area daily and exhaust releases PAHs for atmospheric-water interaction processes (Vento and Dachs, 2007) before settling onto the lake. The PAH levels in front of the outlet (Station 3) are lower than at both inlets (Stations 1 and 4). This might be due to degradation processes occuring in open surface water and acceleration by direct sunlight which can degrade PAHs faster (Jacobs *et al.*, 2008). The lower PAH level at the outlet areas of the lake compared to the inlets might also be due to their accumulation and sinking rates into bottom sediments along the way to the outlets.

The PAHs concentrations in surface water of Lake Perdana range from 322 (Station 5) to 1296 ng L^{-1} (Station 2) (Table 2.11b). However, the highest PAH concentration is not found at the lake inlet (Station 4). This suggests that the surrounding park is not the main contributor of PAHs to the lake surface water. The highest concentration of PAHs is at Station 2 which is located in the middle of the lake. The wide variation of PAH concentrations around the lake suggests that dry deposition of pollutants from the atmosphere has lead to an input of PAHs to the lake surface water. Atmospheric fall- out is known to be an important route of introduction of pollutants into water bodies (Vento and Dachs, 2007). In addition, the location of the lake in the middle of Kuala Lumpur supports this possibility for an atmospheric origin because PAHs were also detected in the city atmosphere (Omar *et al.*, 2002).

Table 2.11 (a) Concentration of PAHs in surface water of Lake Taman Jaya (ng L^{-1})

	Lake Taman Jaya (Concentration; ng L^{-1})									
Compounds	**Station 1**	**Station 2**	**Station 3**	**Station 4**	**Station 5**	**Station 6**	**Max**	**Min**	**Average**	**TEQ^{carc}**
Acenaphthylene	125.1	61.3	51.6	52.9	nd	56.2	125.1	nd	57.9	0.0580
Acenaphthene	127.1	63.3	53.7	54.3	66.2	55.6	127.1	53.7	70.0	0.0700
Fluorene	124.6	60.0	51.0	52.2	nd	53.9	124.6	nd	57.0	0.0570
Phenanthrene	143.7	74.9	66.8	121.1	77.2	68.1	143.7	66.8	92.0	0.0920
Anthracene	nd	nd	52.5	55.9	64.2	nd	64.2	nd	28.8	0.2880
Fluoranthene	134.6	72.9	58.9	64.6	71.9	64.8	134.6	58.9	77.9	0.0780
Pyrene	137.8	67.7	55.6	59.0	69.4	58.5	137.8	55.6	74.7	0.0750
Benz[a]anthracene	nd	60.4	71.0	nd	nd	72.8	72.8	nd	34.0	3.4000
Chrysene	131.0	79.6	52.3	71.3	83.1	57.3	131.0	52.3	79.1	0.7910
Benzo[b]fluoranthene	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.0000
Benzo[k]fluoranthene	nd	nd	nd	nd	65.0	60.8	65.0	nd	21.0	2.1000
Benzo[a]pyrene	nd	nd	nd	nd	nd	57.9	57.9	nd	9.7	9.6600
Indeno[1,2,3- cd]pyrene	124.7	63.9	54.2	55.0	66.3	54.7	124.7	54.2	69.8	69.8000
Dibenz[a,h]anthracene	136.3	nd	57.3	55.3	nd	57.6	136.3	nd	51.1	0.5110
Benzo[g,h,i]perylene	nd	nd	nd	nd	nd	67.9	67.9	nd	11.3	1.1300
Total	1185.0	604.0	624.8	641.5	563.3	785.9	1185.0	563.3	734.1	88.1000

nd: not detected

Table 2.11 (b) Concentration of PAHs in surface water of Lake Perdana (ng L^{-1})

	Lake Perdana (Concentration; ng L^{-1})								
Compounds	**Station 1**	**Station 2**	**Station 3**	**Station 4**	**Station 5**	**Max**	**Min**	**Average**	**TEQ^{carc}**
Acenaphthylene	42.6	98.3	54.0	30.9	30.3	98.3	30.3	51.2	0.0510
Acenaphthene	49.2	101.6	54.1	35.7	32.4	101.6	32.4	54.6	0.0550
Fluorene	40.9	97.7	53.0	29.2	30.0	97.7	29.2	50.1	0.0500
Phenanthrene	56.4	109.2	60.5	41.3	39.3	109.2	39.3	61.3	0.0610
Anthracene	42.4	99.2	53.9	30.4	31.0	99.2	30.4	51.4	0.5140
Fluoranthene	45.6	102.0	57.4	32.7	34.8	102.0	32.7	54.5	0.0540
Pyrene	42.5	99.5	54.9	30.5	32.2	99.5	30.5	51.9	0.0520
Benz[a]anthracene	nd	98.1	53.4	nd	30.2	98.1	nd	36.3	3.6300
Chrysene	41.4	98.5	53.7	35.2	30.7	98.5	30.7	51.9	0.5190
Benzo[b]fluoranthene	nd	nd	nd	nd	nd	nd	nd	nd	0.0000
Benzo[k]fluoranthene	41.3	97.9	nd	nd	nd	97.9	nd	27.8	2.7800
Benzo[a]pyrene	nd	nd	nd	nd	nd	nd	nd	nd	0.0000
Indeno[1,2,3- cd]pyrene	41.7	98.5	nd	30.9	nd	98.5	nd	34.2	34.2000
Dibenz[a,h]anthracene	43.2	97.9	54.4	nd	31.4	97.9	nd	45.4	0.4540
Benzo[g,h,i]perylene	40.9	97.7	nd	29.6	nd	97.7	nd	33.6	3.3600
Total	528.0	1296.1	549.2	326.3	322.2	1296.1	322.2	604.4	45.8000

nd: not detected

Among all PAH target compounds, benzo[b]fluoranthene was not detected in the surface waters of both lakes. In addition, benzo[a]pyrene was also not detected in the surface water samples of Lake Perdana. The concentrations of individual PAHs in these two lakes are less than 1000 ng L^{-1}, suggesting that they are not severely contaminated with PAHs (Guo *et al.*, 2007). However, comparing PAH concentrations in surface waters of these with other lakes studied worldwide, it is observed that the PAHs in surface water of Lakes Taman Jaya and Perdana are highest (Table 2.12). Also, comparing the PAH composition based on ring numbers shows that 3 ring PAHs are the most abundant in both lakes (42 % and 45 % respectively) followed by those with 4 ring (36 % and 32 % respectively). In both lakes, 5 and 6 ring PAHs are equal (11 % for Lake Taman Jaya and 15 % for Lake Perdana respectively) (refer Fig. 2.5a,b).

In Lake Taman Jaya, only PAHs with 5 ring are not detected at some station (Station 2) while for Lake Perdana, PAHs with 5 ring are not detected at Station 4 while PAHs with 6 ring are not detected at Stations 3 and 5.

Table 2.12 Some PAH concentrations in surface waters, SPM and surface sediments worldwide

Sample	Location	Surface Area (km^2)	Mean Depth (Max. Depth, m)	No. of PAHs	Concentration ($ng\ L^{-1}$; $ng\ g^{-1}$ dry weight)[a]	Reference
Water	Lake Taman Jaya, Kuala Lumpur	0.0286	3	15	563- 1185	This study
	Lake Perdana, Kuala Lumpur	0.1295	3	15	322- 1296	This study
	Lake Temsah, Suez Canal, Egypt	8	10	16	52460- 3393000	Ali *et al.* (2006)
	Lake Maggiore, Italy	212	177 (370)	16	2.93	Olivella (2006)
	Lake Redo´, Pyrenees	0.24	73	23	0.409	Vilanova *et al.* (2001)
	Lake Gossenkollesee, Alps	0.017	9.9	23	0.536	Vilanova *et al.* (2001)
	Lake Esthwaite water, UK	1	6.4 (15.5)	12	91.3	Gevao *et al.* (1998)
	Lake Superior, USA	82103	na	13	5.76	Baker and Eisenreich (1990)
SPM	Lake Taman Jaya, Kuala Lumpur	0.0286	3	15	927- 2798	This study
	Lake Perdana, Kuala Lumpur	0.1295	3	15	1209- 2920	This study
	Lake Taihu, China	2338	1.9	16	3370-7531	Qiao *et al.* (2012)
	Lake Maggiore, Italy	212	177 (370)	16	0.584	Olivella (2006)
	Lake Redo´, Pyrenees	0.24	73	23	0.323	Vilanova *et al.* (2001)
	Lake Gossenkollesee, Alps	0.017	9.9	23	0.542	Vilanova *et al.* (2001)

Surface Sediment	Lake Taman Jaya, Kuala Lumpur	0.0286	3	15	149- 237	This study
	Lake Perdana, Kuala Lumpur	0.1295	3	15	99.4- 278	This study
	Lake Sagamore, Adirondack Park, NY, USA	0.68	10.5 (22.9)	17	2900	Tan & Heit (1981)
	Lake Woods, Adirondack Park, NY, USA	0.247	3.5 (10.1)	17	11000	Tan & Heit (1981)
	Lake Michigan, USA	57780	82 (282)	28	1300- 3500	Simcik *et al.* (1996)
	Lake Taihu, China	2338	1.9	16	1207- 4754	Qiao *et al.* (2006)
	Lake Nansi, China	1266	1.46	16	160- 32600	Li *et al.* (2009)
	Lake Baiyangdian, China	366	(9)	16	101.3- 322.8	Hu *et al.* (2010)

[a] Concentration of water in ng L^{-1}; concentrations of SPM and sediment in ng g^{-1} dry weight
na: not available

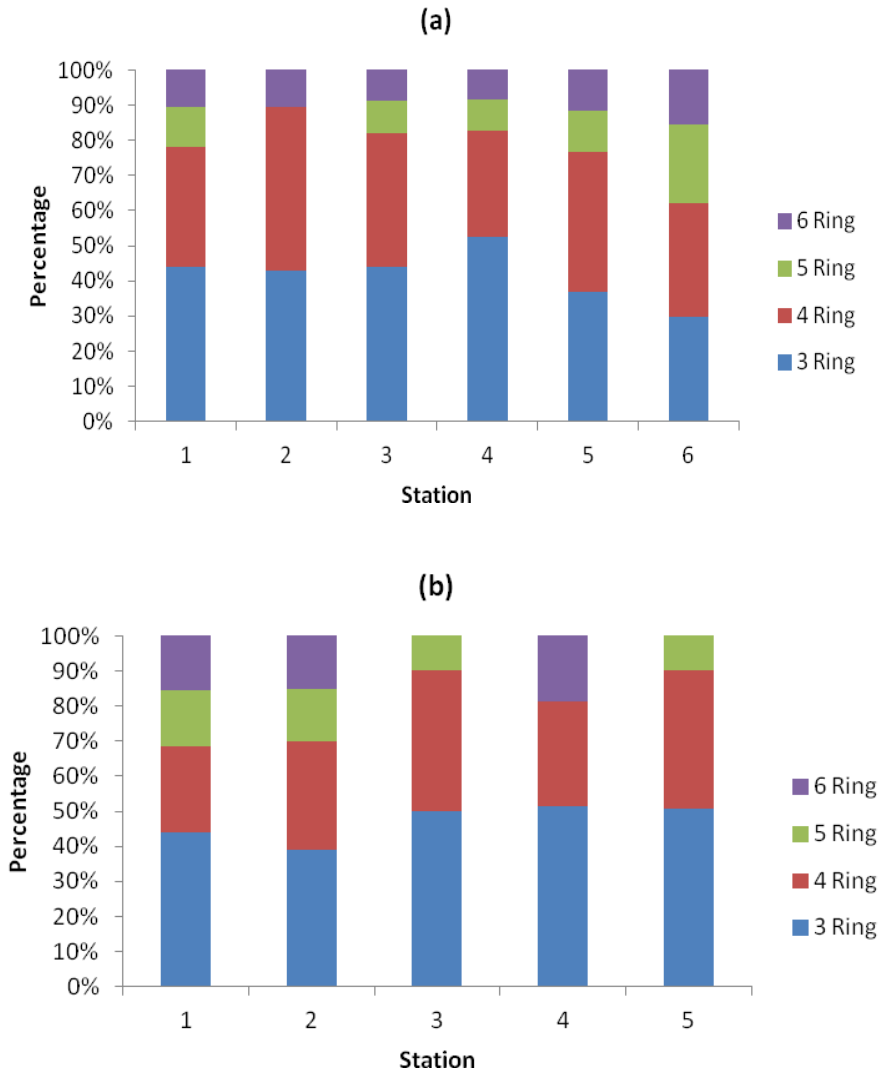

Figure 2.5 Percentage of PAH concentrations based on their number of rings in surface water of (a) Lake Taman Jaya (b) Lake Perdana (3- rings: acenaphthylene, acenaphthene, fluorene, phenanthrene and anthracene; 4- rings: fluoranthene, pyrene, benz[a]anthracene and chrysene; 5- rings: benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene and dibenzo[a,h]anthracene; 6- rings: indeno[1,2,3- cd]pyrene and benzo[g,h,i]perylene).

2.3.2 Concentration of PAHs in SPM

The PAH concentrations of SPM for both lakes (Table 2.13a,b) are different from those in surface waters. The total concentrations of PAHs in SPM of Lake Taman Jaya range from 927 to 2799 ng g^{-1} dry weight (mean 1775 ng g^{-1} dry weight). The highest concentration is at Station 2 and lowest at Station 6 and the water inlet (Stations 1 and 4). This suggests that

the atmospheric deposition may be the main contributor of PAHs to SPM of the lake, and run- off from adjacent residential areas drains into the lake elevating PAH concentrations in the surface water. Furthermore, the SPM content of the lake can be considered as comparatively low (23.3- 32.3 mg L^{-1}, Table 2.1) (Guo *et al.*, 2007).

PAH concentration in SPM of Lake Taman Jaya (Table 2.13a) are not significantly different from the values recorded for Lake Perdana (1210- 2921 ng g^{-1} dry weight, mean 1852 ng g^{-1} dry weight) ($p > 0.05$). The highest PAH concentration in SPM of Lake Perdana is at Station 5 (2921 ng g^{-1} dry weight), located in one of the main boat passages, and the lowest is at Station 2 in the lake center (1210 ng g^{-1} dry weight, Table 2.13b). The PAHs average value in SPM of Lake Perdana is 1852 ng g^{-1} with SPM content lower than Lake Taman Jaya (8.10- 14.4 mg L^{-1} (Table 2.1)). This also suggests an atmospheric input as the main PAH source.Table 2.12 shows the comparison between PAHs of SPM in this study with other studies worldwide.

Table 2.13 (a) Concentration of PAHs in SPM of Lake Taman Jaya (ng g^{-1} dry weight)

	Lake Taman Jaya (Concentration; ng g^{-1} dry weight)									
Compounds	**Station 1**	**Station 2**	**Station 3**	**Station 4**	**Station 5**	**Station 6**	**Max**	**Min**	**Average**	**TEQ^{carc}**
Acenaphthylene	13.3	160.7	24.4	89.8	136.7	33.1	160.7	13.3	76.3	0.0760
Acenaphthene	nd	215.0	149.4	137.0	137.1	33.9	215.0	nd	112.1	0.1120
Fluorene	463.8	145.8	264.3	79.2	133.9	31.2	463.8	31.2	186.4	0.1860
Phenanthrene	31.6	176.3	8.4	108.9	179.3	67.5	179.3	8.4	95.3	0.0950
Anthracene	nd	nd	50.6	nd	nd	nd	50.6	nd	8.4	0.0840
Fluoranthene	112.0	344.5	276.1	220.2	346.7	195.1	346.7	112.0	249.1	0.2490
Pyrene	224.0	481.4	271.6	366.4	590.0	322.0	590.0	224.0	375.9	0.3760
Benz[a]anthracene	19.3	169.3	22.3	79.3	136.5	34.5	169.3	19.3	76.9	7.6900
Chrysene	7.1	152.6	6.7	92.1	143.2	38.5	152.6	6.7	73.4	0.7340
Benzo[b]fluoranthene	24.4	169.7	23.4	104.1	157.5	nd	169.7	nd	79.9	7.9900
Benzo[k]fluoranthene	23.5	161.1	8.0	89.8	146.5	32.2	161.1	8.0	76.9	7.6900
Benzo[a]pyrene	55.0	181.9	39.3	109.0	171.0	33.5	181.9	33.5	98.3	98.3000
Indeno[1,2,3- cd]pyrene	15.9	nd	nd	91.6	nd	35.2	91.6	nd	23.8	23.8000
Dibenz[a,h]anthracene	296.3	289.5	101.1	91.4	191.7	37.8	296.3	37.8	168.0	1.6800
Benzo[g,h,i]perylene	42.0	150.9	nd	83.2	140.4	32.9	150.9	nd	74.9	7.4900
Total	1328.0	2798.9	1245.8	1741.9	2610.7	927.4	2798.9	927.4	1775.5	157.0000

nd: not detected

Table 2.13 (b) Concentration of PAHs in SPM of Lake Perdana (ng g^{-1} dry weight)

	Lake Perdana (Concentration; ng g^{-1} dry weight)								
Compounds	**Station 1**	**Station 2**	**Station 3**	**Station 4**	**Station 5**	**Max**	**Min**	**Average**	**TEQ^{carc}**
Acenaphthylene	103.5	67.9	179.4	61.0	214.5	214.5	61.0	125.3	0.1250
Acenaphthene	110.3	66.9	178.6	63.9	287.2	287.2	63.9	141.4	0.1410
Fluorene	95.9	61.5	173.3	50.1	201.0	201.0	50.1	116.4	0.1160
Phenanthrene	173.4	72.3	180.7	65.4	213.4	213.4	65.4	141.0	0.1410
Anthracene	nd	141.5	260.1	142.9	342.7	342.7	nd	177.4	1.7700
Fluoranthene	269.8	212.0	295.7	308.3	419.9	419.9	212.0	301.2	0.3010
Pyrene	357.8	346.4	439.9	469.0	551.5	551.5	346.4	432.9	0.4330
Benz[a]anthracene	107.7	71.6	nd	61.0	217.4	217.4	nd	91.5	9.1500
Chrysene	nd	nd	nd	nd	nd	nd	nd	nd	0.0000
Benzo[b]fluoranthene	nd	nd	nd	nd	nd	nd	nd	nd	0.0000
Benzo[k]fluoranthene	nd	nd	nd	nd	nd	nd	nd	nd	0.0000
Benzo[a]pyrene	125.9	89.0	203.6	76.3	241.1	241.1	76.3	147.2	147.0000
Indeno[1,2,3- cd]pyrene	nd	nd	200.0	nd	nd	200.0	nd	40.0	40.0000
Dibenz[a,h]anthracene	112.0	80.8	nd	79.5	232.6	232.6	nd	101.0	1.0100
Benzo[g,h,i]perylene	nd	nd	184.7	nd	nd	184.7	nd	36.9	3.6900
Total	1456.3	1209.8	2295.9	1377.4	2921.0	2921.0	1209.8	1852.1	204.0000

nd: not detected

Individual PAHs are present at low concentrations in all stations (< 1000 ng g^{-1}). Compositional pattern of PAHs based on its number of rings are shown in Fig. 2.6 (a) and (b). The results show that PAHs with 4 rings are the most abundant PAHs with 44 % in Lake Taman Jaya and 45 % in Lake Perdana followed by PAHs with 3 ring (27 % and 38 % respectively), 5 ring (24 % and 14 % respectively) and 6 ring (6 % and 4 % respectively). PAHs with 3 to 6 benzene rings are recorded in all stations of Lake Taman Jaya except Station 3 which recorded the absence of 6 rings PAHs. For Lake Perdana, PAHs with 6 rings are only recorded in Station 3 while PAHs with 3 to 5 rings were recorded in all stations.

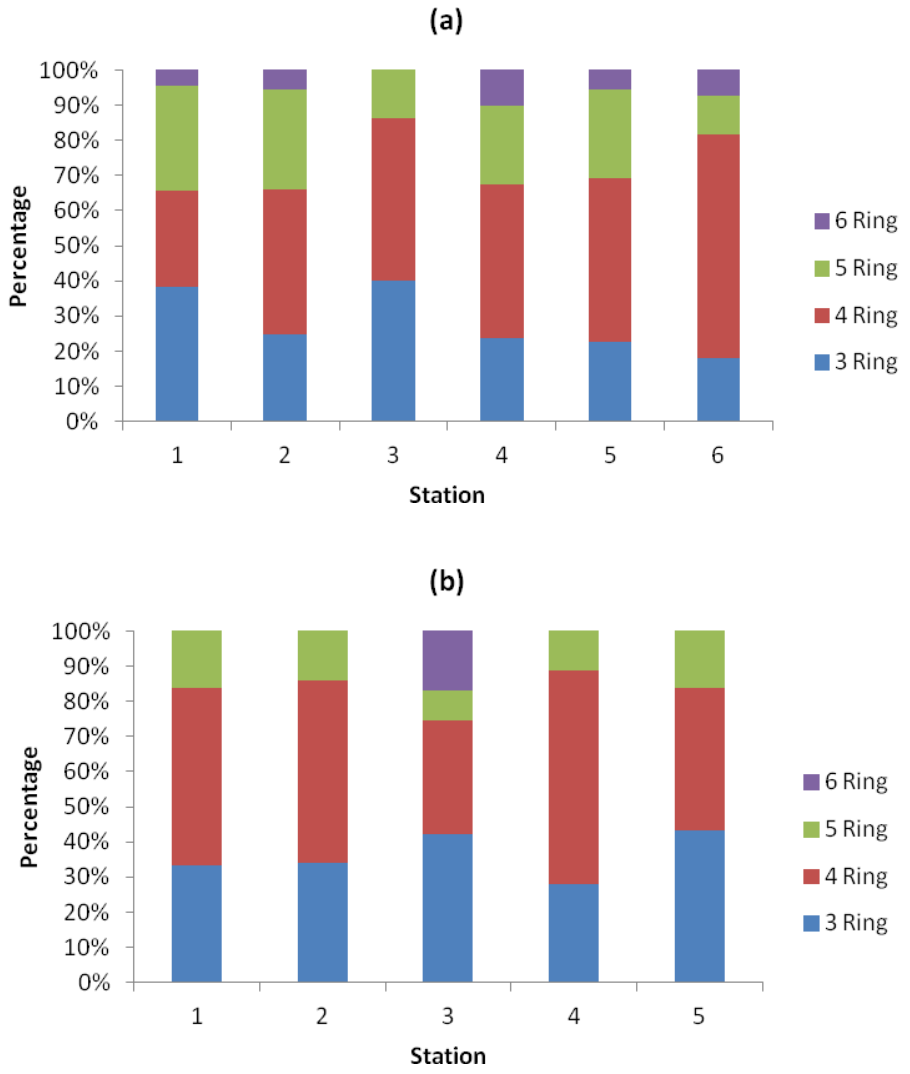

Figure 2.6 Percentage of PAHs concentration based on their number of rings in SPM of (a) Lake Taman Jaya (b) Lake Perdana (3- rings: acenaphthylene, acenaphthene, fluorene, phenanthrene and anthracene; 4- rings: fluoranthene, pyrene, benz[a]anthracene and chrysene; 5- rings: benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene and dibenzo[a,h]anthracene; 6- rings: indeno[1,2,3- cd]pyrene and benzo[g,h,i]perylene).

2.3.3 PAHs in surface sediments

The PAH concentrations in surface sediment samples from both lakes are given in Table 2.14 (a) and (b). The total concentration for Lake Taman Jaya samples (Table 2.14a) ranges from 149 (Station 3) to 237 ng g^{-1} dry weight (Station 4) with a mean of 205 ng g^{-1} dry weight, and Lake Perdana has a total concentration ranging from 99.4 (Station 3) to 278 ng g^{-1} dry weight (Station 4), mean 163 ng g^{-1} dry weight (Table 2.14b). These PAH levels are

lower than in the other media of both lakes, which might be due to sediment resuspension and current erosion during flood. It may also be from sediment removal during lake maintenance processes. Comparing with the concentrations of PAHs in sediments studied worldwide, PAHs is studied lakes are still at low level (Table 2.12).

Table 2.14 (a) Concentration of PAHs in surface sediments of Lake Taman Jaya (ng g^{-1} dry weight)

	Lake Taman Jaya (Concentration; ng g^{-1} dry weight)									
Compounds	**Station 1**	**Station 2**	**Station 3**	**Station 4**	**Station 5**	**Station 6**	**Max**	**Min**	**Average**	**TEQ^{carc}**
Acenaphthylene	92.4	31.2	20.6	53.8	30.2	20.8	92.4	20.6	41.5	0.0420
Acenaphthene	8.5	6.4	1.0	9.0	16.4	1.0	16.4	1.0	7.04	0.0070
Fluorene	0.9	7.8	5.2	2.4	7.1	5.9	7.8	0.9	4.9	0.0050
Phenanthrene	3.6	16.6	14.5	9.9	20.4	16.4	20.4	3.6	13.6	0.0140
Anthracene	2.0	nd	2.4	2.8	nd	nd	2.8	nd	1.2	0.0120
Fluoranthene	3.6	19.7	17.5	15.8	20.2	23.6	23.6	3.6	16.8	0.0170
Pyrene	6.5	17.8	18.4	17.7	24.0	22.8	24.0	6.5	17.9	0.0180
Benz[a]anthracene	11.2	7.7	6.2	13.7	7.4	8.6	13.7	6.2	9.2	0.9150
Chrysene	16.5	16.1	14.1	21.6	20.0	16.6	21.6	14.1	17.5	0.1750
Benzo[b]fluoranthene	16.5	27.0	6.8	17.4	8.2	9.8	27.0	6.8	14.3	1.4300
Benzo[k]fluoranthene	15.6	nd	nd	15.1	nd	nd	15.6	nd	5.1	0.5110
Benzo[a]pyrene	8.0	6.6	7.2	9.47	6.4	9.7	9.7	6.4	7.9	7.9000
Indeno[1,2,3- cd]pyrene	11.6	9.6	7.3	11.1	10.1	12.5	12.5	7.3	10.4	10.4000
Dibenz[a,h]anthracene	16.6	3.1	nd	12.8	2.6	2.8	16.6	nd	6.3	0.0630
Benzo[g,h,i]perylene	16.0	41.4	27.9	24.3	41.3	38.2	41.4	16.0	31.5	3.1500
Total	229.5	211.1	149.1	236.8	214.1	188.8	236.8	149.1	204.9	24.6000

nd: not detected

Table 2.14 (b) Concentration of PAHs in surface sediments of Lake Perdana (ng g^{-1} dry weight)

	Lake Perdana (Concentration; ng g^{-1} dry weight)								
Compounds	**Station 1**	**Station 2**	**Station 3**	**Station 4**	**Station 5**	**Max**	**Min**	**Average**	**TEQ^{carc}**
Acenaphthylene	34.1	54.2	46.1	33.0	59.6	59.6	33.0	45.4	0.0450
Acenaphthene	1.6	1.3	2.2	18.3	5.0	18.3	1.3	5.7	0.0060
Fluorene	1.5	2.5	1.1	1.0	0.9	2.5	0.9	1.4	0.0010
Phenanthrene	3.6	6.8	3.6	3.6	9.7	9.7	3.6	5.5	0.0050
Anthracene	2.1	1.2	1.7	3.2	2.1	3.2	1.2	2.0	0.0200
Fluoranthene	6.8	9.6	7.6	5.6	16.7	16.7	5.6	9.3	0.0090
Pyrene	7.3	7.3	4.9	8.6	6.9	8.6	4.9	7.0	0.0070
Benz[a]anthracene	10.2	2.9	3.0	15.5	2.9	15.5	2.9	6.9	0.6870
Chrysene	16.0	5.6	4.8	23.8	5.9	23.8	4.8	11.2	0.1120
Benzo[b]fluoranthene	19.4	5.7	4.5	30.9	5.9	30.9	4.5	13.3	1.3300
Benzo[k]fluoranthene	17.5	4.2	4.1	29.4	4.7	29.4	4.1	12.0	1.2000
Benzo[a]pyrene	8.5	3.4	3.9	13.5	4.6	13.5	3.4	6.8	6.7800
Indeno[1,2,3- cd]pyrene	14.3	4.0	3.6	25.6	4.4	25.6	3.6	10.4	10.4000
Dibenz[a,h]anthracene	17.8	0.9	0.9	34.8	0.9	34.8	0.9	11.1	0.1100
Benzo[g,h,i]perylene	20.2	9.7	7.6	31.3	9.7	31.3	7.6	15.7	1.5700
Total	180.8	119.2	99.4	278.0	139.8	278.0	99.4	163.4	22.2000

Anthracene (at Stations 2, 5 & 6), benzo[k]fluoranthene (at Stations 2,3,5 & 6) and dibenz[a,h]anthracene (at Station 3) are not detected in surface sediments of Lake Taman Jaya. On the other hand, all individual composition of PAHs is detected in surface sediments of Lake Perdana with concentrations less than 60.0 ng g^{-1}.

Based on the number of rings (Fig. 2.7a,b), PAH with 3, 4, 5 and 6 rings are detected in all stations in both lakes. In Lake Taman Jaya, the composition of PAH with 3 rings are the most abundant (33 %) followed by PAH with 4 rings (30 %), 6 rings (20 %) and five rings (16 %). Meanwhile, in Lake Perdana, PAH with 3 rings (37 %) are also the most abundant PAH in it surface sediments. It is followed by 5 ring PAHs (26 %), 4 ring (21 %) and 6 ring (16 %). The concentration of 5 rings PAH are very high at Station 4 of Lake Perdana in comparison to other stations suggesting the sources may came from the water inlet of the lake.

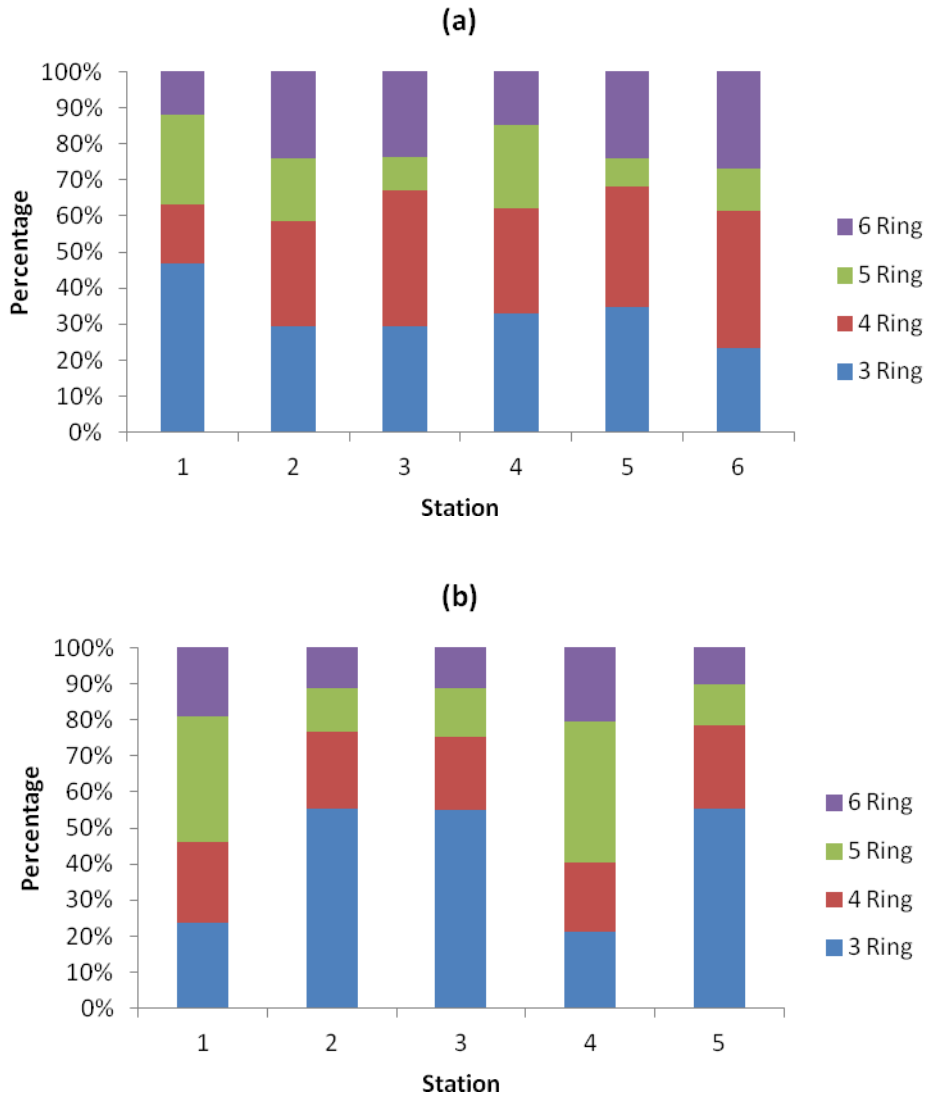

Figure 2.7 Percentage of PAHs concentration based on their number of rings in surface sediments of (a) Lake Taman Jaya (b) Lake Perdana (3- rings: acenaphthylene, acenaphthene, fluorene, phenanthrene and anthracene; 4- rings: fluoranthene, pyrene, benz[a]anthracene and chrysene; 5- rings: benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene and dibenzo[a,h]anthracene; 6- rings: indeno[1,2,3- cd]pyrene and benzo[g,h,i]perylene).

2.3.4 Comparison between the distribution of PAHs in surface water, SPM and surface sediments

The comparison between concentration of PAHs in surface water, SPM and surface sediments are done by assessing the distribution of their low and high molecular weight PAHs. According to Choudhary and Routh (2010), low molecular weight (LMW) PAHs consist of PAH with 2 and 3 rings while high molecular weight PAHs (HMW) consist of PAH with 4 rings or more. Other than its application for distribution analysis,

comparison between LMW and HMW PAHs are also useful for discerning the petrogenic or pyrolgenic origin of PAHs. PAHs can be produced naturally or through anthropogenic activities. However, the main contributor of PAHs in our environment is anthropogenic activities and these activites may be classified into two types, namely, pyrogenic (incomplete combustion of fossil fuels) and petrogenic (discharge of petroleum- related materials) (Huang *et al.*, 2003). Pyrogenic PAHs are generally characterized by the dominance of high molecular mass (4- 6 rings) PAH over those with low (2- 3 rings) molecular mass (Witt and Trost, 1999).

As shown in Fig. 2.8 (a) and (b), surface water of Lake Taman Jaya and Lake Perdana are dominated by HMW PAHs at 58 % and 56 %, respectively. The LMW/ HMW PAHs ratio were calculated with the resulting mean value is 0.7 in Lake Taman Jaya and 0.8 in Lake Perdana. According to Wise *et al.* (1988), the higher the LMW/ HMW ratio, the higher the prevalence of petrogenesis. Thus, the result suggests that PAHs in surface waters of both lakes originated from the same sources which are from pyrogenic activities.

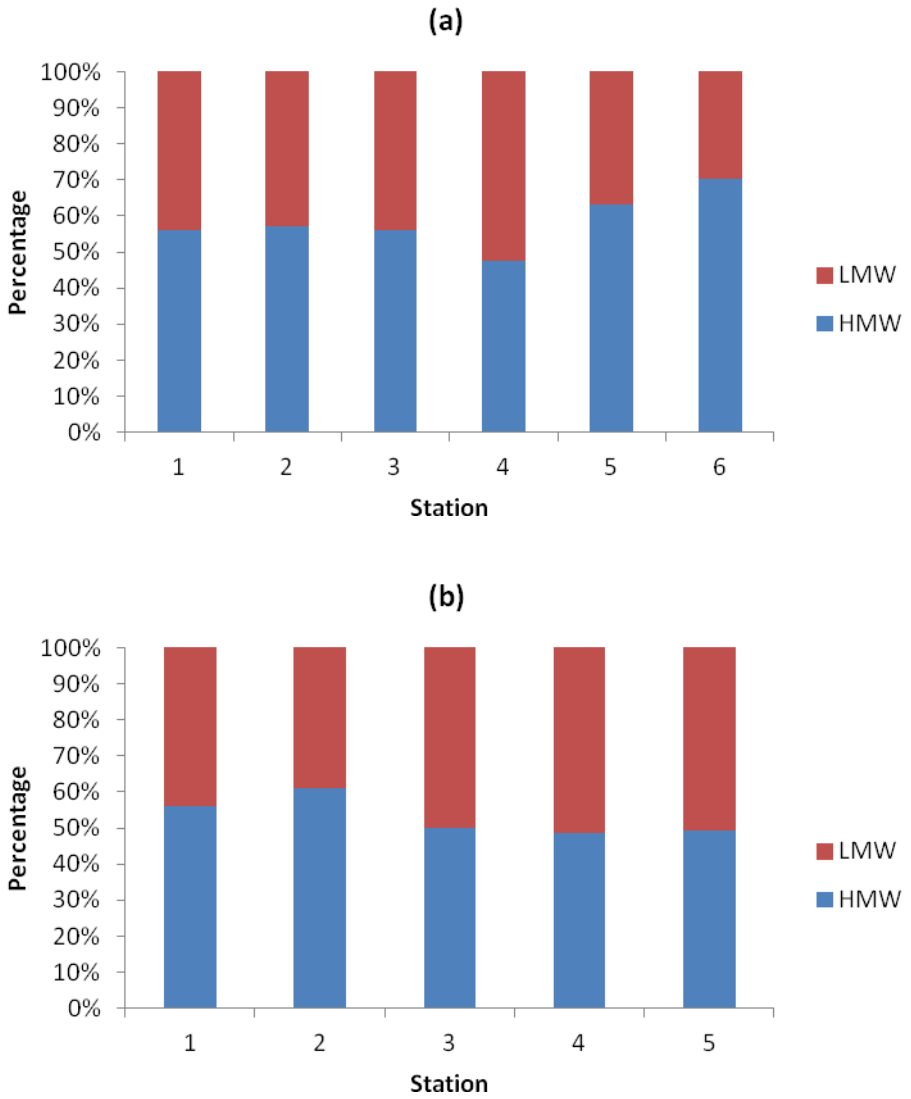

Figure 2.9 Percentage of low molecular weight (LMW) PAHs against high molecular weight (HMW) PAHs in the surface water of (a) Lake Taman Jaya and (b) Lake Perdana, Kuala Lumpur (LMW includes: acenaphthylene, acenaphthene, phenanthrene, and anthracene; while HMW includes: fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[g,h,i]perylene and indeno[123-cd]pyrene).

On the other hand, SPM of Lake Taman Jaya and Lake Perdana are also dominated by HMW PAHs with 73 % and 62 %, respectively. The resulting mean ratio of LMW/ HMW PAHs in Lake Taman Jaya and Lake Perdana are 0.4 and 0.6, respectively. This in turn suggests that the origin of PAHs in SPM of both lakes are from pyrogenic sources. The results are illustrated in Fig. 2.9 (a) and (b).

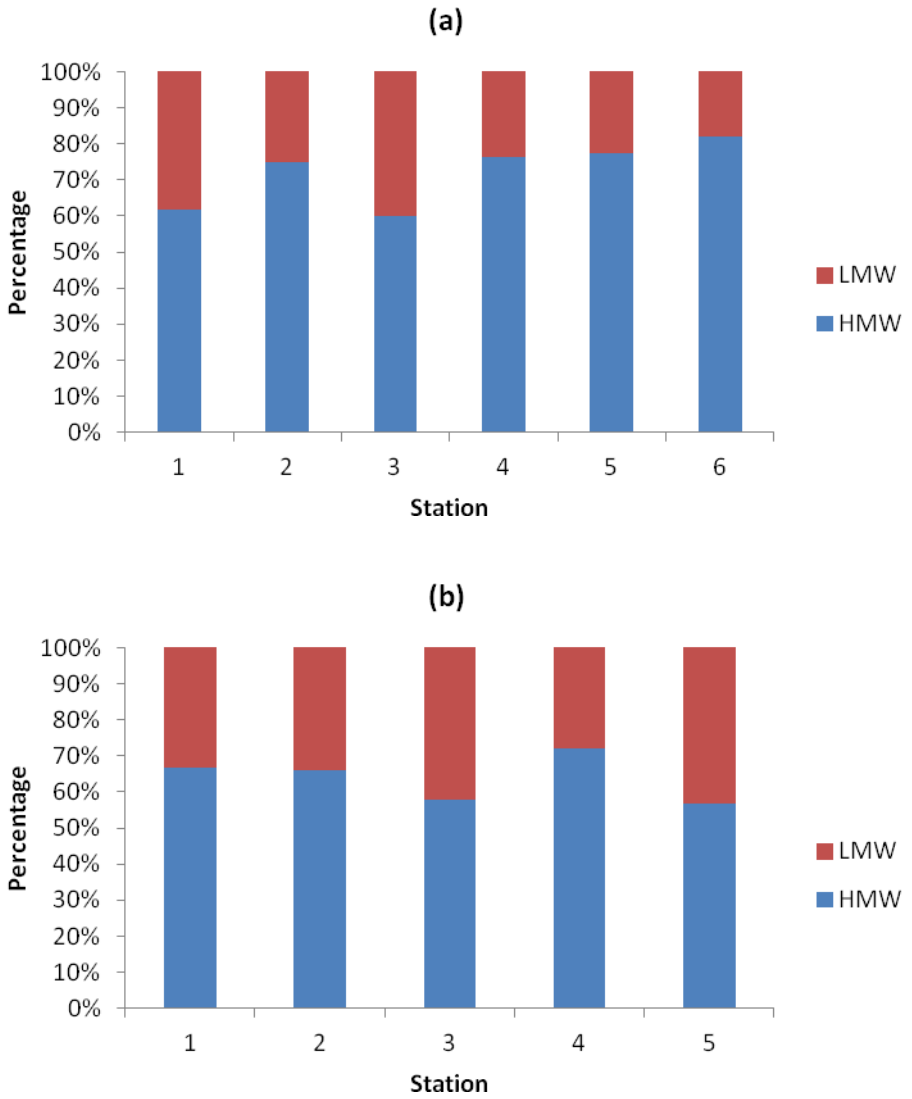

Figure 2.10 Percentage of LMW PAHs against HMW PAHs in the SPM of (a) Lake Taman Jaya and (b) Lake Perdana, Kuala Lumpur (LMW and HMW include the list in Fig. 2.8 legend).

Domination of HMW PAHs in surface sediments of both lakes (except for Station 2, 3 and 5 of Lake Perdana whereby the HMW percentage are 55 %, 55 % and 55 %, respectively) (Fig. 2.10) suggest that mostly, PAHs in Lake Taman Jaya and Lake Perdana originated from both pyrogenic and petrogenic sources. PAHs composition in sediments of urban lakes in this study are believed to support the theory that PAHs with high molecular weight will dominate the distribution of PAHs in sediments since low molecular weight PAH will gradually decreased by degradation and adsorption process

and only those PAHs with high molecular weight are more resistant to degradation can survive to reach sediments (Guo *et al.*, 2007).

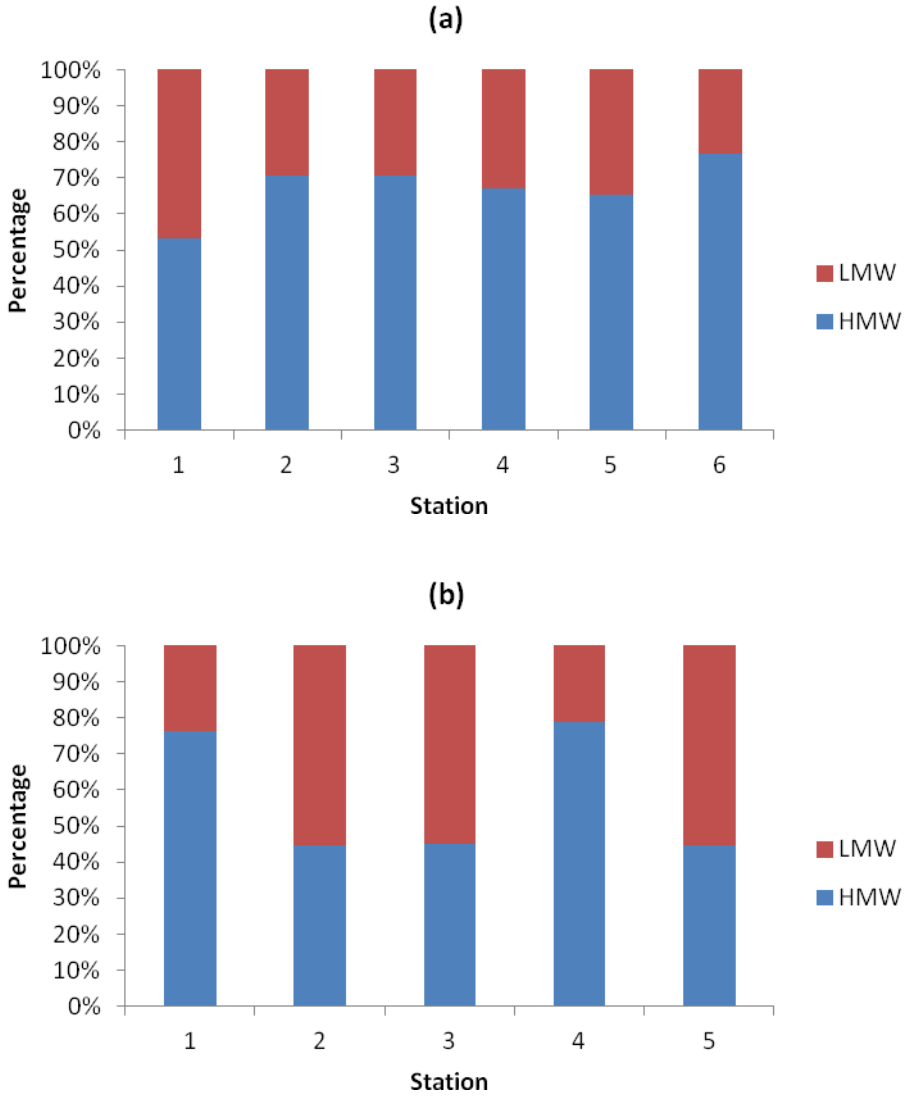

Figure 2.11 Percentage of LMW PAHs against HMW PAHs in Surface Sediments of (a) Lake Taman Jaya and (b) Lake Perdana, Kuala Lumpur (LMW and HMW include the list in Fig. 2.8 legend).

PAHs in SPM and surface sediments are not reported in the same unit as PAHs in surface water. The comparison between PAH concentrations in SPM and surface sediments for both lakes suggests that SPM traps a greater amount of PAHs compared to surface sediments (Fig. 2.11a,b). This might be due to SPM as the first medium in contact with the sources (atmosphere and water inlet) and its higher on content. Besides,

degradation and desorption taking place during sinking of SPM into the sediments, PAH levels gradually decrease especially for the LMW PAHs (Guo *et al.*, 2007).

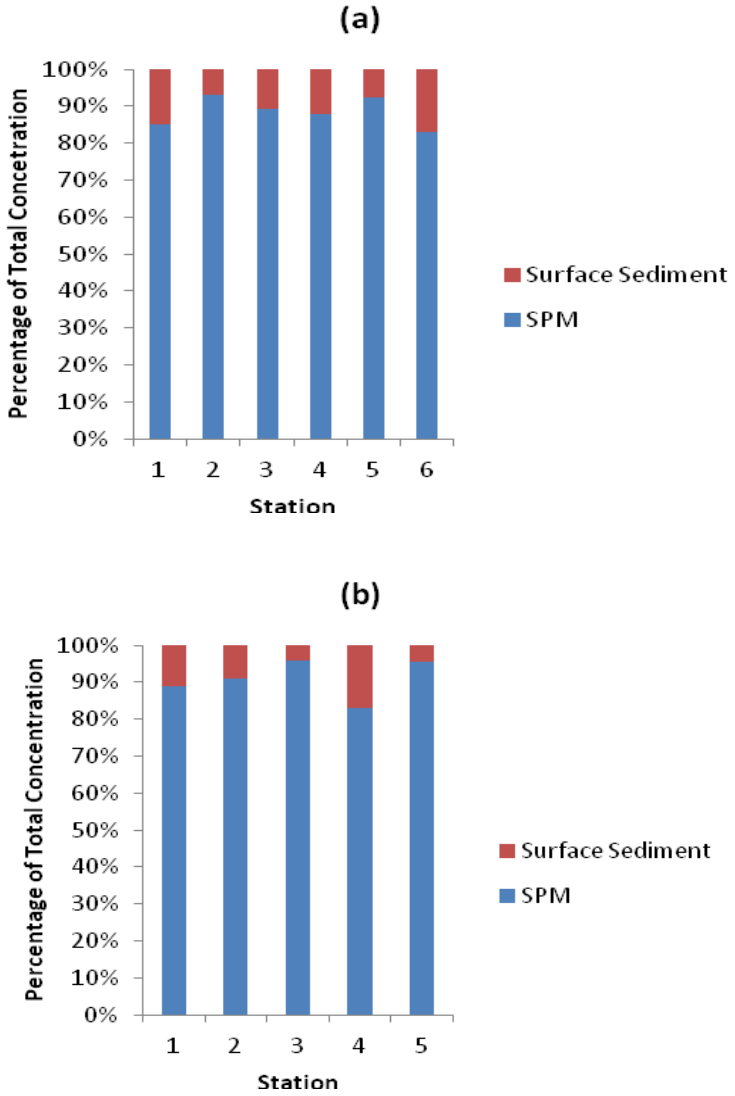

Figure 2.12 Comparison between PAHs in surface sediments and SPM as percentage for both lakes for (a) Lake Taman Jaya and (b) Lake Perdana

2.3.5 Sources of PAHs

A further contribution to the knowledge of PAH origin is given by analyzing the phenantrene/ anthracene and fluoranthene/ pyrene ratio on surface water, SPM and surface sediments of both lakes. The ratio of Phen and Anth is extensively used to infer the nature of PAH pollution (Soclo *et al.*, 2000; Magi *et al*., 2002). Phenanthrene and anthracene are structural isomers; phenanthrene is more thermodynamically stable than

anthracene, therefore, in petrogenic PAH pollution the Phen/ Anth ratio is very high, while high temperatures during the combustion process help the formation of anthracene and a lowering of the Phen/ Anth ratio. Because of the differences in reactivity and solubility of these two pairs of isomers, their respective ratios are not expected to remain constant and cannot, therefore, provide a better picture of the progress of PAHs from their origin through environmental transport, to deposition in sediments. Fluoranthene and pyrene, both with a mass of 202, have the greatest range in stability and hence are good as indicators of thermodynamic vs. kinetic (e.g. petroleum vs. combustion) effects.

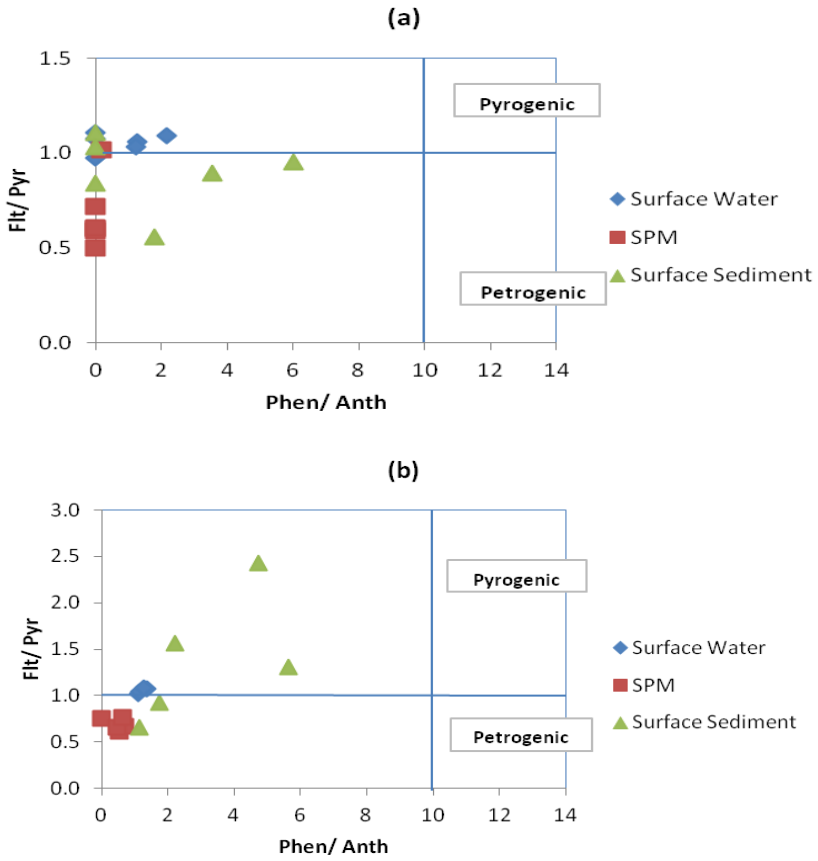

Figure 2.13 Plot of the isomeric ratios Flt/ Pyr vs. Phen/ Anth to assess sources for (a) Lake Taman Jaya and (b) Lake Perdana

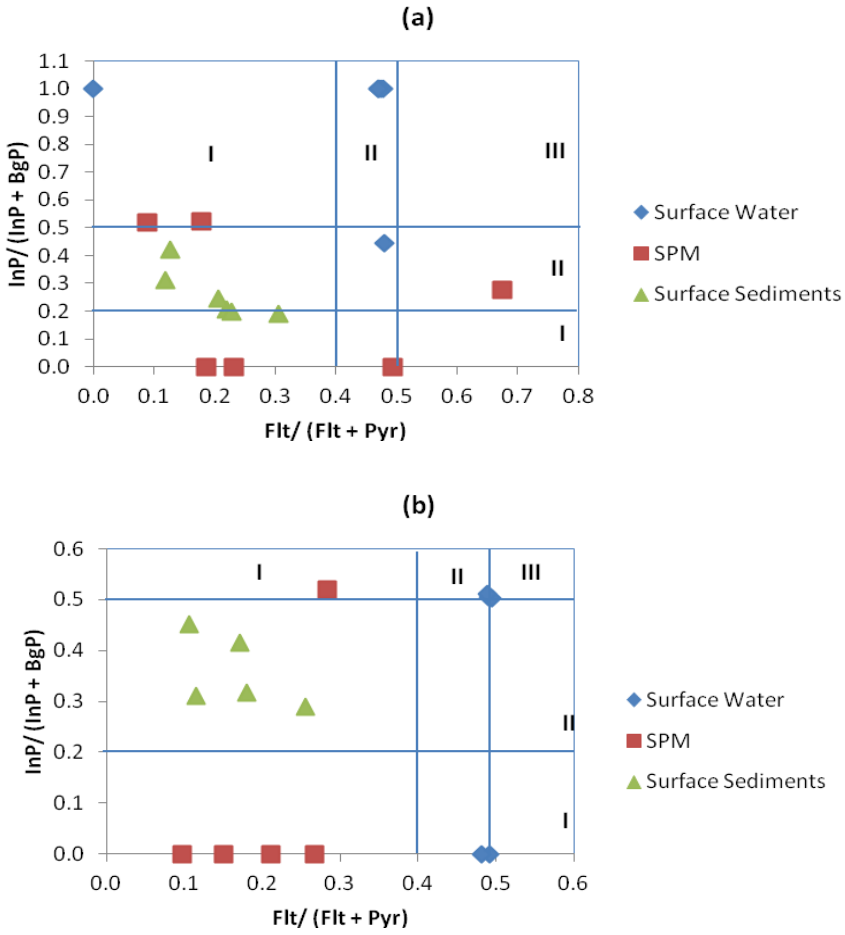

Figure 2.14 Plot of the isomeric ratios Flt/ (Flt + Pyr) vs InP/ (InP + BgP) to identify petroleum/ combustion transition point of PAHs in (a) Lake Taman Jaya and (b) Lake Perdana (Indicator: **I**= Petroleum; **II**= Petroleum Combustion; **III**= Biomass and Coal Combustion).

Correlation between Flt/ Pyr over Phen/ Anth can be used to identify sources of PAHs. A Flt/ Pyr ratio below 1 indicates petrogenic sources, while a ratio above 1 means a pyrogenic origin (Yunker *et al.*, 2002; Doong and Lin, 2004). On the other hand, Phen/ Anth ratios lower than 10 show combustion processes, whereas values > 10 are from petroleum input or diagenetic (Baumard *et al.*, 1998). The Flt/ (Flt + Pyr) ratio of 0.4 illustrates the source is from petroleum/combustion transition point, while < 0.4 corresponds to petroleum pollution and < 0.5 is from biomass or coal combustion (Baumard *et al.*, 1998; Yunker *et al.*, 2002). An InP/ (InP + BgP) ratio < 0.2 suggests a petroleum input/ spillage, > 0.5 represents biomass or coal combustion, while a ratio between 0.2 and 0.5 indicates petro- chemical fuel combustion (Yunker *et al.*, 2002; Luo *et al.*, 2005).

Based on Fig. 2.12 and 2.13, surface waters of both Lakes Taman Jaya and Perdana shows that pyrogenic sources have a great impact on the concentration of PAHs with transition point indicating at petroleum pollutant stage. For PAHs in SPM, it is shown that petrogenic activities have a greater contribution compared to pyrogenic activities in Lake Taman Jaya. The transition point shows that the level of PAHs is influenced by mixed contribution of petroleum discharge, petro- chemical combustion by vehicles, and such. The concentrations are also influenced by biomass or coal combustion. Meanwhile, SPM in Lake Perdana shows that the petroleum pollutant is the most significant sources of its PAHs. On the other hand, surface sediments in both lakes show the influence of both pyrogenic and petrogenic activities on their distribution which probably came from petroleum discharge and petro- chemical fuel combustion.

2.3.6 Risk assessment

There is no recommended value for PAHs in fresh water by the USEPA (1990 a,b) for water quality assessment. However, comparing individual PAH values as priority toxic pollutants with the limit for priority pollutants in water plus organisms (0.0044 mg L^{-1}), shows that the concentration of individual PAHs in these lake surface waters are high, including the hazardous compounds. HMW PAHs, especially benzo[a]pyrene, benzo[k]fluoranthene and indeno[1,2,3- cd]pyrene are known carcinogens (Huang *et al.*, 2003). The PAH concentrations in SPM are also high in both lakes, suggesting a continuous input to the lakes. The PAHs on SPM can affect organisms or example, indeno[1,2,3- cd]pyrene and benzo[k]fluoranthene can cause tumors in mice through various types of exposure (USEPA, 1990a). Benzo[k]fluoranthene is also mutagenic to bacteria (USEPA, 1990b).

Ecological risk assessment of sediments can be used to assess the effects of contaminants in aquatic environments (Agarwal *et al.,* 2006; Guo *et al.,* 2011). There are many guidelines that can be applied to assess PAH pollution in surface sediments.

Some of the effect- based guidelines are given in Table 2.15 (Long *et al.*, 1995; Yi and Lee, 1999). A value of ER- L at 4000 ng g^{-1} (total PAH concentration) indicates that sediments may cause harmful effects towards biota. However, the total PAH concentrations in surface sediments of both lakes are lower than 4000 ng g^{-1}. Since the concentrations of individual PAHs for this study ranged from non- detectable to 92.4 ng g^{-1} in Lake Taman Jaya and from 0.87 to 60 ng g^{-1} in Lake Perdana, it can be concluded that all PAHs are lower than the limit in the guidelines except for acenaphthylene based on the guideline proposed by Long *et al.* (1995) (Table 2.15), or TEL by FDEP (Table 2.15) (all Stations in both lakes), and also ER- L by NOAA (Table 2.15) (Stations 1 and 4 for Lake Taman Jaya, Stations 2, 3 and 5 for Lake Perdana). The concentrations of dibenz[a,h]anthracene (Table 2.14a,b) at Stations 1 and 4 of both Lake Taman Jaya and Lake Perdana are also found higher than the TEL guideline set up by FDEP, which has a high probability of causing acute biological damage.

Table 2.15 Standard pollution criteria for PAHs in sediment matrix (ng g^{-1})

Compound	**Long *et al.* (1995)**		**NOAA***		**FDEP***	
	ER- L	**ER- M**	**ER- L**	**ER- M**	**TEL**	**PEL**
Naphthalene	160	2100	160	2100	34.6	391
Acenaphthene	44	640	16	500	6	88.9
Acenaphthylene	16	500	44	640	5.87	128
Fluorene	19	540	19	540	21.2	144
Phenanthrene	240	1500	240	1500	86.7	544
Anthracene	85	1100	853	1100	46.9	245
Fluoranthene	600	5100	600	5100	113	1494
Pyrene	665	2600	665	2600	153	1398
Benz[a]anthracene	261	1600	261	1600	74.8	693
Chrysene	384	2800	384	2800	108	846
Benzo[b]fluoranthene	na	na	na	na	na	na
Benzo[k]fluoranthene	na	na	na	na	na	na
Benzo[a]pyrene	430	1600	430	1600	88.8	763
Dibenz[a,h]anthracene	63	260	63.4	260	6.20	135
Benzo[g,h,i]perylene	na	na	na	na	na	na
Indeno[1,2,3- cd]pyrene	na	na	na	na	na	na
Total	4022	44792	4000			

* NOAA: National Oceanic and Atmospheric Administration; FDEP: Florida Department of Environmental Regulation; ER- L: Effective Range Low; ER- M: Effective Range Medium; TEL: Threshold Effect Level; PEL: Probable Effect Level; na: not available (Yi and Lee, 1999)

2.3.7 Sediments toxicity

Some PAHs are well known for their carcinogenicity. Savinov *et al.* (2003) grouped PAHs to calculate the total concentration of potential carcinogenic PAHs (CPAH) and included benz[a]anthracene, benzofluoranthenes, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, and dibenz[a,h]anthracene. Based on this sediment toxicity assessment from the total CPAH concentration, the Lake Taman Jaya sediments values varied from 26.7 to 95.0 ng g^{-1} dry weight Lake Perdana from 19.4 to 149 ng g^{-1}. Here, we used TEFs to estimate the toxic level of the surface sediments by calculating toxic equivalent factors ($TEFs^{carc}$) from their PAH contents. $TEFs^{carc}$ are often used to assess the toxicological risk of PAHs in sediments rather than in surface waters and SPM. Peters *et al.* (1999) stated that only benzo[a]pyrene in a PAH group has adequate toxicologic data to derive a carcinogen is potential factor among other potential carcinogenic PAHs. $TEFs^{carc}$ were used here to compare carcinogenicity of PAHs relative to benzo[a]pyrene as used by Nadal *et al.* (2004). Table 2.16 shows TEF values as adopted from Tsai *et al.*, 2004.

Table 2.16 TEF values

Compound	TEF Value
acenaphthylene	0.001
acenaphthene	0.001
fluorene	0.001
phenanthrene	0.001
anthracene	0.01
fluoranthene	0.001
pyrene	0.001
benz[a]anthracene	0.1
chrysene	0.01
benzo[b]fluoranthene	0.1
benzo[k]fluoranthene	0.1
benzo[a]pyrene	1
dibenz[a,h]anthracene	1
benzo[g,h,i]perylene	0.01
indeno[1,2,3- cd]pyrene	0.1

All values were then converted into one toxic concentration for the mean values for both lakes using the corresponding $TEFs^{carc}$. The calculation was based on the equation:

$$\mathbf{TEQ^{carc} = \sum_i C_i \times TEF_i^{carc}} \qquad \text{Equation 2.5}$$

The values of Total B[a]P toxicity quivalents (TEQ^{carc}) determined for surface sediments of Lake Taman Jaya and Lake Perdana were 24.6 ng g^{-1} TEQ^{carc} and 22.2 ng g^{-1} TEQ^{carc}, respectively. In comparison with other reports (Qiao *et al.*, 2006; Savinov *et al.*, 2003), the TEQ^{carc} for these surface sediments is low.

2.4 Conclusion

This is first survey of 15 priority PAHs in water, SPM and sediments of two urban recreational lakes in Kuala Lumpur under the influence of city activities. The PAH concentrations have a moderate to high correlation among the sample types. The PAH levels are higher in water and SPM than in surface sediments for both lakes. The high molecular weight PAHs are dominant in both lakes indicating greater influence of pyrogenic activities. However, fingerprint analyses have shown that the PAHs in Lake Taman Jaya and Lake Perdana came from both pyrogenic and petrogenic sources. The assessment of carcinogenic hazard by calculation of TEQ^{carc} for surface sediments of both lakes indicates a low level compared to other world regions. It is suggested that sediments from both lakes can be dredged and disposed without causing harmful effects towards exposed organisms. The benzo[a]pyrene concentrations at all sampling sites do not exceed 4000 ng g^{-1}.

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Chapter 3

Chapter 3

Identification and Characterization of Polycyclic Aromatic Hydrocarbons (PAHs) in Road Side Soils and Road Dusts of Industrial, Commercial and Residential Areas, Kuala Lumpur Malaysia

3.1 Introduction

Cancer has become the second highest cause of death in Malaysia (2008 with 6.9 %, DOS, 2010). Cancer researchers estimated that as many as 2 in 3 cancer cases (67 %) are linked to some type of environmental factor including pollution in air, water, and soil (NCI, 2010). Polycyclic aromatic hydrocarbons (PAHs) are one group of environmental pollutants that have the potential to cause cancer in animals and humans.

PAHs have multiple aromatic rings resulting in their stable molecular structure (Liu *et al.*, 2007), which retards photochemical decomposition rates and biodegradation processes, thus contributing to their persistence in the environment. PAHs are derived from both natural oxidative processes, such as organic matter decomposition, forest wildfires, etc. and incomplete combustion in anthropogenic processes, such as vehicle exhaust, coal combustion, incinerator waste, petroleum spillage, etc. (Liu *et al.*, 2010). The amount of PAHs emitted to the atmosphere increases with decreasing efficiency of the combustion processes (Khan *et al.*, 2008). The most favorable temperatures for PAH production are between 300- 740 $^{\circ}C$ (Moret and Conte, 2000). Urban areas have become the supply region of PAHs in the environment due to their huge utilization of energy sources (Li *et al.*, 2006a). There have been more than 160 PAHs identified in the environment, however, only 16 were listed as priority pollutants by the United States

Environmental Protection Agency (EPA) (Schwarzenbach *et al.*, 1993; Khan *et al.*, 2008).

Vehicle exhaust releases gaseous and particulate matter and has become the main contributor of PAHs and other toxic substances such as trace metals (Nelson *et al.*, 2008). The increasing numbers of vehicles registered every year is worrying as it will increase the levels of toxic substances in the atmosphere which in turn deposit to soils and water bodies with rainfall. In Malaysia, the number of vehicles has increased from 13.8 to 18.0 million between 2004 and 2008 (DOS, 2010), and the amount of rainfall recorded in Kuala Lumpur also seems on the rise (2009 from 136 to 424 mm, and 2010 from 110 to 537 mm; DOM, 2011).

According to Pirjola *et al.* (2010), re- suspension of surface particulate matter (PM) is an important source for road dusts. However, the main mechanisms for road dust emissions are still unidentified. Other than heavy metals, PAHs are also known as major contaminants in road dust (Kose *et al.*, 2008), and are derived from incomplete pyrolysis of organic materials from various urban sources (Hancock *et al.*, 1970; Hassanien and Abdel- Latif, 2008). Studies have shown that traffic density and the rate of deposition do influence the concentration of PAHs in street dust (Edwards, 1989; Jones, 1989; Novotny and Olem, 1994). Earlier studies have reported that the origin of PAHs in road dust were mainly from vehicle exhaust (Takada *et al.*, 1990; Kose *et al.*, 2008), road pavement material or asphalt (Faure *et al.*, 2000; Kose *et al.*, 2008), tire rubber and lubricant oil (Faure *et al.*, 2000). Similar to aerosols, road dust falls out and washes out onto surface water by rain (Hunter *et al.*, 1979; Kumata *et al.*, 2000; Zhao *et al.*, 2009) bringing along the PAHs. The concentrations of PAHs in road dust vary with the distance of its sources (Li *et al.*, 2006b).

On the other hand, for soils, atmospheric deposition is known to be the most common source of pollution (Li *et al.*, 2006b; ATSDR, 1995). Contamination by PAHs

is believed to be greater in urban areas than rural, forest and agricultural regions as the former normally have a higher density of vehicles and most of the PAH emissions are expected to accumulate in surface soil (Agarwal *et al.*, 2009; Tham *et al.*, 2008).

The soil system seems to be a suitable medium to study environmental pollution, as an important long- term repository for PAHs (Wild *et al.*, 1990; Wild and Jones, 1995). Moreover, humans may be exposed more to PAHs through contact with soils rather than air or water (Menzie *et al.*, 1992; Agarwal *et al.*, 2009).

The properties of PAHs and the environmental medium play an important role in determining the fate of PAHs (He *et al.*, 2009). Also, location controls the distribution rather than size- fractions and sampling times (Murakami *et al.*, 2005). Even though studies by Morillo *et al.* (2008) in Sevilla, Spain didn not found any significant differences of PAHs concentration in different land uses (between agricultural, road sides and park soils), other studies such as by Wilcke (2000), Krauss and Wilcke (2003), Wang *et al.* (2008), Baek *et al.* (1991) and Maisto *et al.* (2006) did found that land use of the studied areas have a big impact on the concentration obtained. Wilcke (2000), has reported that the concentration of PAH increased greatly with decreasing distance from the roads due to the reduced vehicular emissions. A study by Krauss and Wilcke (2003) showed that the concentration of PAHs in soils of a garden and industrial areas (> 10 µg kg^{-1}) were eightfold higher than the park soils and agricultural soils (0.64 µg kg^{-1}). Wang *et al.* (2008) in their study in New Orleans found that the highest amounts of PAHs recorded in soils close to the roads was 7,189 µg kg^{-1} which is so much greater than recorded in open spaces (2,404 µg kg^{-1}) that were 10 m away from the roads. Meanwhile, Baek *et al.* (1991) in their study had reported that urban soils near highways were highly contaminated. In Naples, Italy, it has been reported that the total PAHs concentrations were 2- 20 times higher than the park that was located 12 km away (Maisto *et al.*, 2006).

Industrial besides urban areas are locales which are expected to have high PAH concentrations derived from the surrounding industries, such as coal tar plants, coking plants, municipal trash incinerators, etc. PAHs in industrial areas can also be from petroleum refineries (Wcislo *et al.*, 1997; Dong and Lee, 2009). The PAH input from vehicular traffic is also important in industrial areas. On the other hand, commercial and residential areas are expected to have lower PAH concentrations since the expected main sources of the PAHs are just the vehicle emissions.

PAHs enter the human body through the lungs from breathing air, by drinking water and swallowing food with associated soil or dust particles. Also, low molecular weight (volatile) PAHs can be absorbed through the skin but the absorption of high molecular weight (heavy) PAHs is quite limited (ATSDR, 1995; ATSDR, 1990). Accumulations of PAHs in soils can also disrupt food chains and contaminate vegetables (Meharg *et al.*, 1998; Kipopoulou *et al.*, 1999). Soils could input PAHs to the atmosphere and groundwater through leaching, evaporation and migration processes (Bispo *et al.*, 1999; Cousins *et al.*, 1999). Therefore, it is important to know the levels of PAHs in soils and road dusts for risk prevention of harmful effects.

Since there are no previous data focusing on the concentrations of PAHs in the three major types of land uses of Kuala Lumpur (namely industrial, commercial and residential), this study aims to determine the levels of PAHs in road dusts and road side soils, and to assess the potential sources and health risks. These results provide essential reference information to set up a baseline PAH value for future assessments.

3.2 Experimental

3.2.1 Sampling sites

Sampling areas for the collection of road dusts and road side soils samples was selected based on land- used map provided by Kuala Lumpur City Hall (KLCH) (Ref: DBKL/JPI/BPPM/PS/Ogos09/WPKI). Five (5) areas marked as industrial areas in the

KLCH map were chose for this study (Fig. 3.1) and their coordinates are shown in Table 3.1. It includes industrial area in Chan Sow Lin, Kuchai Lama, Segambut and two (2) industrial areas in Kepong. These areas are among the largest industrial areas in Kuala Lumpur and their locations quite far from one another.

As for the commercial areas, six (6) areas were selected as the sampling locations namely Ampang Park, Sultan Ismail, Masjid India, Bukit Bintang, Imbi and Raja Chulan (Fig. and Table 3.2). Three of these locations (Imbi, Sultan Ismail and Raja Chulan) are located in the so- called “Kuala Lumpur Golden Triangle”. It is a famous, large area with towering sky scrapers, shopping malls and world- class hotels dominating the landscape. To ensure precise data obtained to represent PAHs concentrations in road dusts and road side soils of Kuala Lumpur’s commercial areas, sampling was conducted along the streets together with other locations that have also been developed as commercial hub of Kuala Lumpur city.

Six areas (shown in Fig. and Table 3.3) were selected based on the KLCH land use map to represent residential areas in Kuala Lumpur with two locations located near to industrial areas (Segambut and PPR Sg. Besi), two locations near to commercial areas (U-Thant and Kampung Baru) and two locations far from both industrial and commercial areas (Jalan Datuk Sulaiman and Puncak Jalil). Segambut residential area and PPR Sg Besi residential areas are located within 5 km radius from the nearest industrial areas while U- Thant and Kampung Baru are located within 5 km radius from nearby commercial areas. The other two locations have been identified as locations that are far ($\geq$ 5 km) from both industrial and commercial activities.

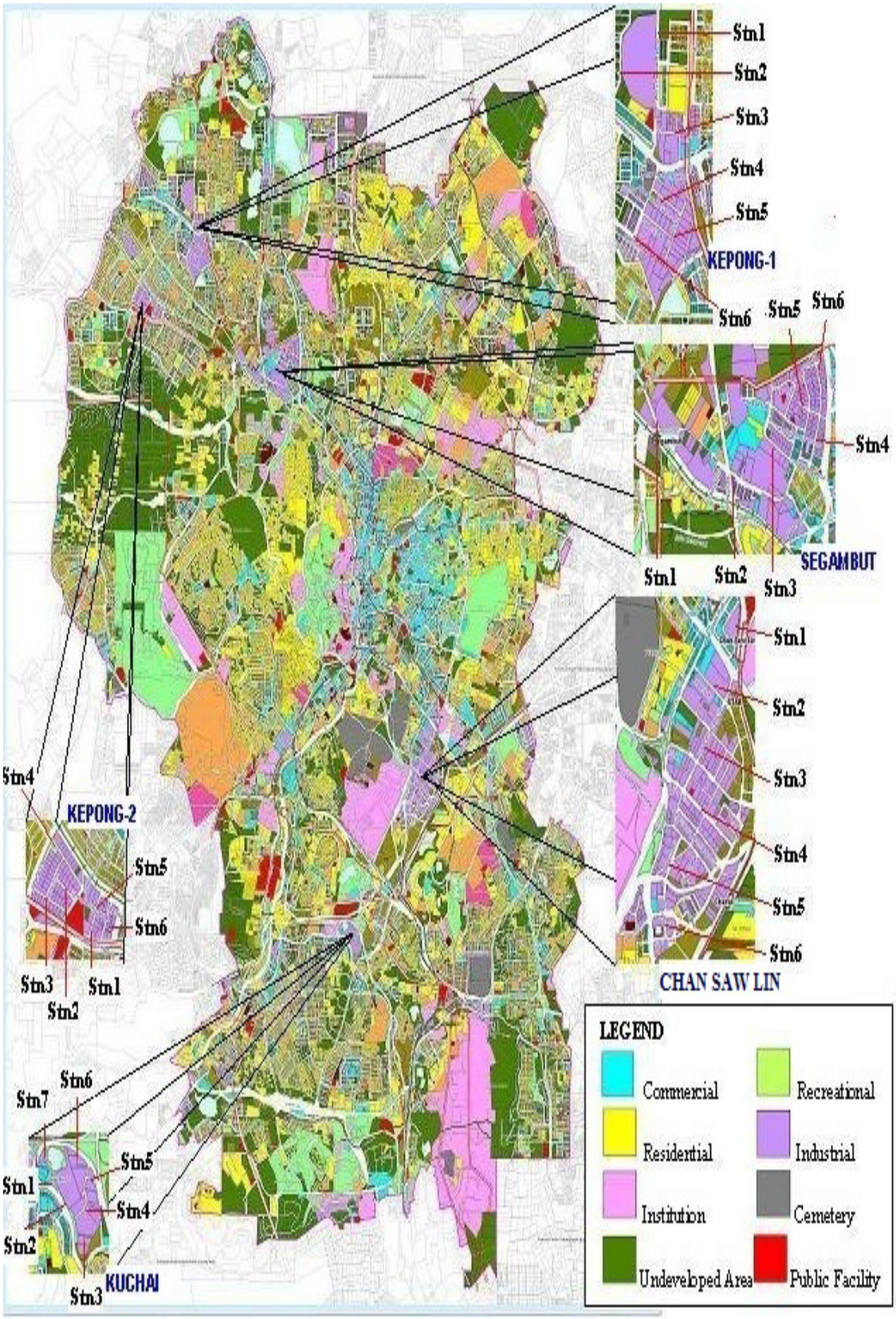


Figure 3.1 Study areas and sampling locations in the Chan Saw Lin, Kuchai, Segambut, Kepong- 1 and Kepong- 2 industrial areas of Kuala Lumpur, Malaysia (Land- used map provided by Kuala Lumpur City Hall, Ref: DBKL/JPI/BPPM/PS/Ogos09/WPKI)

Table 3.1 Coordinates and concentrations of total extracted lipids from road dusts and road side soils of industrial areas of Kuala Lumpur

STATION		COORDINATES		TEL (mg g^{-1})	
				Road Dust	Road Side Soil
Chan Saw Lin	Station 1	N03°07.533'	E101°.42.632'	2.43	2.04
	Station 2	N03°07.290'	E101°.42.709'	6.57	14.51
	Station 3	$N03^{0}$07.126'	$E101^{0}$42.582'	13.71	0.72
	Station 4	N03°06.886'	E101°42.735'	7.02	22.51
	Station 5	N03°07.004'	E101°42.827'	2.87	6.52
	Station 6	N03°07.310'	E101°42.575'	9.27	21.01
Kuchai	Station 1	N03°05.583'	E101°41.650'	9.67	0.50
	Station 2	N03°05.617'	E101°41.577'	8.25	0.55
	Station 3	N03°05.592'	E101°41.595'	41.11	0.55
	Station 4	N03°05.557'	E101°41.517'	9.84	1.05
	Station 5	N03°05.407'	E101°41.567'	6.97	1.99
	Station 6	N03°05.358'	E101°41.589'	6.80	1.71
	Station 7	N03°05.316'	E101°41.596'	15.6	0.99
Segambut	Station 1	N03°11.420'	E101°40.628'	9.60	5.47
	Station 2	N03°11.280'	E101°40.741'	13.9	11.01
	Station 3	N03°11.168'	E101°40.510'	10.11	7.46
	Station 4	N03°11.346'	E101°40.375'	9.69	2.27
	Station 5	N03°11.060'	E101°40.588'	9.25	6.80
	Station 6	N03°11.006'	E101°40.408'	10.11	3.10
Kepong- 1	Station 1	N03°12.424'	E101°39.138'	8.14	4.54
	Station 2	N03°12.330'	E101°39.262'	4.31	2.71
	Station 3	N03°12.188'	E101°39.187'	6.74	0.72
	Station 4	N03°12.258'	E101°39.016'	2.32	6.63
	Station 5	N03°12.378'	E101°39.020'	7.29	0.44
	Station 6	N03°12.301'	E101°39.141'	6.35	3.71
Kepong- 2	Station 1	N03°11.688'	E101°38.499'	1.94	3.82
	Station 2	N03°11.729'	E101°38.597'	4.37	0.66
	Station 3	N03°11.768'	E101°38.660'	2.71	0.44
	Station 4	N03°11.582'	E101°38.432'	6.64	1.44
	Station 5	N03°11.651'	E101°38.565'	5.53	3.15
	Station 6	N03°11.752'	E101°38.413'	2.21	8.24

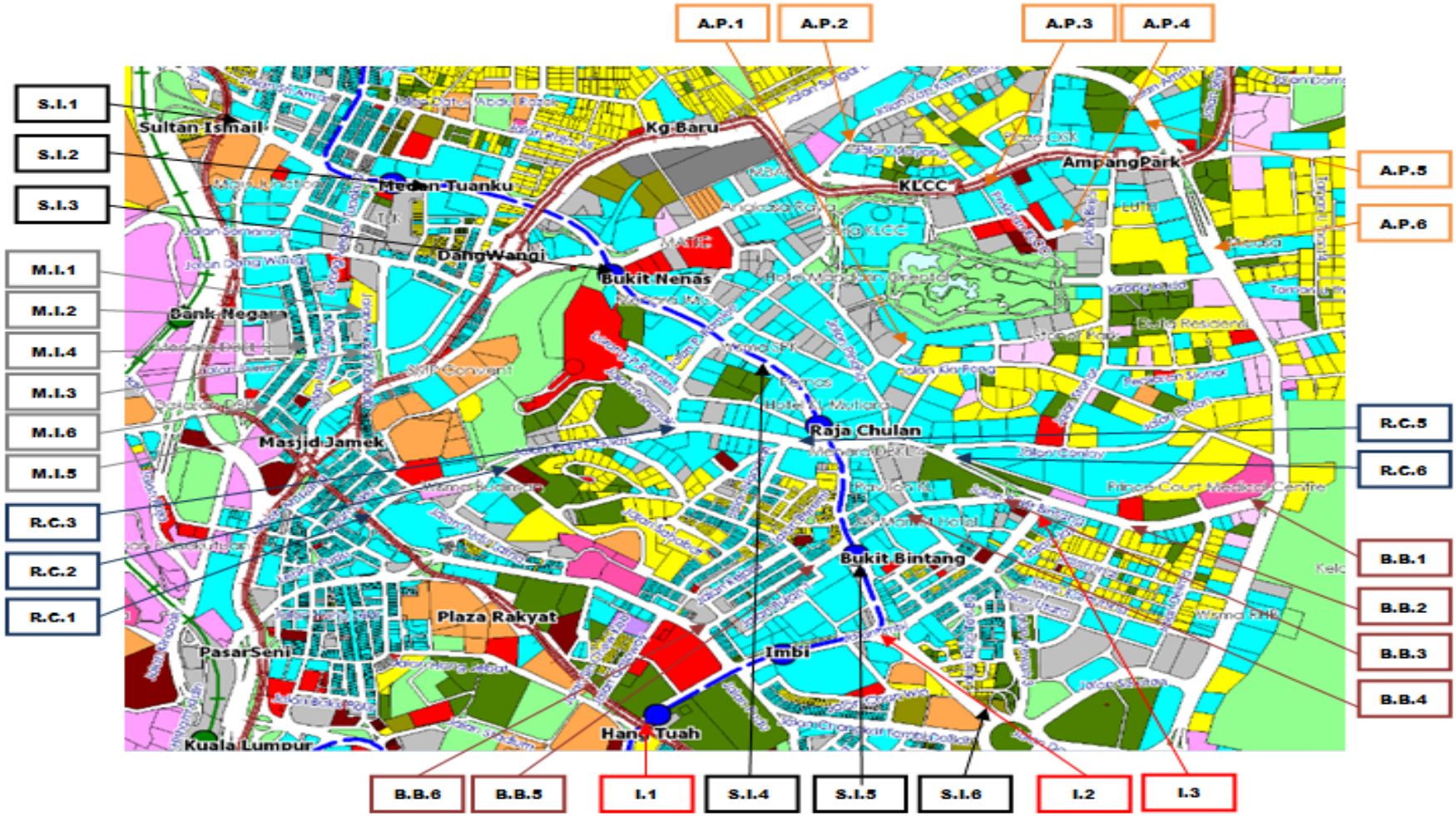


Figure 3.2 Study areas and sampling locations in the Ampang Park, Sultan Ismail, Masjid India, Bukit Bintang, Imbi and Raja Chulan commercial areas of Kuala Lumpur, Malaysia (Ref: DBKL/JPI/BPPM/PS/Ogos09/WPKI)

Table 3.2 Coordinates and concentrations of total extracted lipids from road dusts and road side soils of commercial areas of Kuala Lumpur

STATION		COORDINATES		TEL (mg g^{-1})	
				Road Dust	Road Side Soil
Ampang Park	Station 1	N03°09.431'	E101°42.393'	7.58	3.38
	Station 2	N03°09.465'	E101°.42.641'	6.20	5.81
	Station 3	N03°09.516'	E101°42.362'	30.10	0.83
	Station 4	N03°09.606'	E101°43.108'	19.17	4.27
	Station 5	N03°09.396'	E101°42.521'	15.79	0.39
	Station 6	N03°09.169'	E101°42.816'	11.08	2.93
Sultan Ismail	Station 1	N03°09.670'	E101°41.740'	6.64	0.55
	Station 2	N03°09.548'	E101°42.178'	8.20	0.50
	Station 3	N03°09.268'	E101°42.446'	11.41	1.66
	Station 4	N03°09.082'	E101°42.614'	6.37	2.27
	Station 5	N03°08.729'	E101°42.688'	10.97	0.94
	Station 6	N03°08.443'	E101°42.893'	13.62	2.16
Masjid India	Station 1	N03°09.401'	E101°41.759'	8.19	2.71
	Station 2	N03°09.239'	E101°41.846'	10.74	3.05
	Station 3	N03°09.164'	E101°41.847'	20.98	4.37
	Station 4	N03°09.411'	E101°41.693'	22.12	3.43
	Station 5	N03°09.201'	E101°41.775'	9.08	0.78
	Station 6	N03°09.436'	E101°41.793'	11.07	1.00
Bukit Bintang	Station 1	N03°08.868'	E101°43.289'	7.20	2.49
	Station 2	N03°08.892'	E101°42.974'	11.85	1.49
	Station 3	N03°08.835'	E101°42.832'	3.93	0.83
	Station 4	N03°08.613'	E101°42.479'	8.74	3.32
Imbi	Station 1	N03°08.841'	E101°42.888'	12.46	0.39
	Station 2	N03°08.638'	E101°42.734'	10.58	3.26
	Station 3	N03°08.588'	E101°42.545'	9.91	1.44
Raja Chulan	Station 1	N03°08.909'	E101°41.917'	9.58	6.70
	Station 2	N03°09.012'	E101°42.187'	8.59	4.65
	Station 3	N03°09.017'	E101°42.513'	9.74	1.05
	Station 4	N03°09.005'	E101°42.724'	7.58	1.05
	Station 5	N03°08.966'	E101°42.809'	11.90	9.02

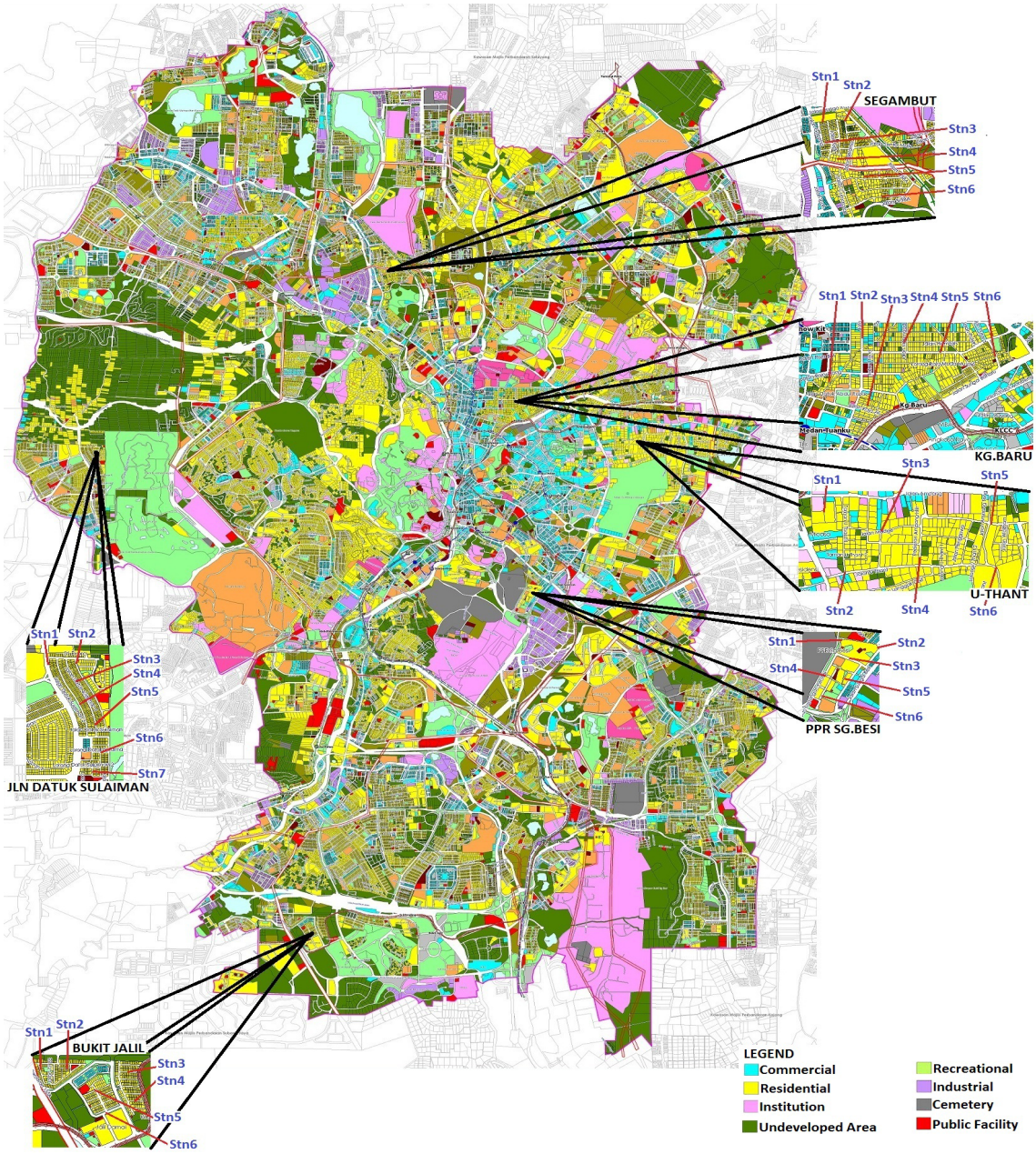


Figure 3.3 Study areas and sampling locations in the Segambut, PPR Sg. Besi, Jln Datuk Sulaiman, Kampung Baru, U- Thant and Puncak Jalil residential areas of Kuala Lumpur, Malaysia (Ref: DBKL/JPI/BPPM/PS/Ogos09/WPKI)

Table 3.3 Coordinates and concentrations of total extracted lipids from road dusts and road side soils of residential areas of Kuala Lumpur

STATION		COORDINATES		TEL (mg g^{-1})	
				Road Dust	Road Side Soil
U- Thant	Station 1	N03°09.836'	E101°43.726'	23.69	5.04
	Station 2	N03°09.888'	E101°.43.406'	8.47	2.93
	Station 3	N03°09.903'	E101°.43.172'	11.63	1.44
	Station 4	N03°09.773'	E101°43.401'	4.59	9.84
	Station 5	N03°09.776'	E101°43.609'	11.40	0.22
	Station 6	N03°09.841'	E101°43.850'	19.43	1.05
Kg. Baru	Station 1	N03°09.661'	E101°42.242'	15.88	1.77
	Station 2	N03°09.990'	E101°42.226'	4.60	0.50
	Station 3	N03°09.876'	E101°42.771'	6.81	1.94
	Station 4	N03°10.045'	E101°42.499'	8.25	6.69
	Station 5	N03°09.925'	E101°42.440'	8.42	0.17
	Station 6	N03°09.856'	E101°42.228'	9.42	0.88
Segambut	Station 1	N03°10.929'	E101°40.697'	4.70	0.66
	Station 2	N03°11.040'	E101°40.728'	8.47	10.08
	Station 3	N03°11.065'	E101°40.643'	5.47	2.71
	Station 4	N03°10.965'	E101°40.746'	13.18	0.72
	Station 5	N03°10.985'	E101°40.633'	3.38	1.60
	Station 6	N03°11.057'	E101°40.583'	45.58	2.77
PPR Sg Besi	Station 1	N03°06.424'	E101°42.319'	15.06	8.64
	Station 2	N03°06.457'	E101°42.274'	3.49	0.44
	Station 3	N03°06.649'	E101°42.303'	10.85	6.70
	Station 4	N03°06.667'	E101°42.377'	4.65	3.49
	Station 5	N03°06.539'	E101°42.373'	6.47	0.94
	Station 6	N03°06.497'	E101°42.299'	17.87	1.66
Puncak Jalil	Station 1	N03°01.741'	E101°40.644'	22.15	0.17
	Station 2	N03°01.883'	E101°40.625'	10.80	0.06
	Station 3	N03°01.787'	E101°40.784'	10.35	0.06
	Station 4	N03°01.981'	E101°41.046'	18.88	0.06
	Station 5	N03°01.760'	E101°40.984'	7.48	1.99
	Station 6	N03°01.987'	E101°40.756'	16.06	9.79
Jln. Datuk Sulaiman	Station 1	N03°09.354'	E101°37.717'	1.94	0.17
	Station 2	N03°08.793'	E101°37.866'	9.52	5.09
	Station 3	N03°08.855'	E101°37.777'	10.02	1.27
	Station 4	N03°09.071'	E101°37.837'	7.92	0.39
	Station 5	N03°09.133'	E101°37.770'	13.78	0.55
	Station 6	N03°09.202'	E101°37.824'	20.32	0.17
	Station 7	N03°08.906'	E101°37.889'	2.10	5.09

3.2.2 Reagent, glassware and apparatus

Reagent types, handling methods, reagent preparations, glassware and apparatus for this experiment are as per described in Chapter 2 (Section 2.2.2).

3.2.3 Standards

As described in Chapter 2 (Section 2.2.3).

3.2.4 Sampling and sample preparation

3.2.4.1 Road side soil

Surface road side soils (0– 5 cm depth) were taken with a stainless steel soil auger, pooled and homogenized to provide a composite sample. The uppermost plant cover, twigs and stones were removed before transfer to 500 ml glass bottles (pre- rinsed with MeOH and DCM), covered with aluminum foil and capped. All samples were preserved at 4 °C for transfer to the laboratory. In laboratory, the samples were kept in refrigerator at -20 °C. The samples were taken out, freeze- dried, grinded, sieved, homogenized and left in a dessicator before extraction.

3.2.4.2 Road dusts

On the other hand, road dusts were collected using sweeping tools (brush and steel dustpan) and stored in pre- washed glass bottles, also covered with aluminum foil and capped. The samples were also preserved at $4^{\circ C}$ till for transfer and in the laboratory, the sample bottles were stored at -20 °C before freeze- dried overnight. The samples were then sieved through a 300 µm mesh and left in a dessicator before extraction.

3.2.5 Extraction

After coning and quartering, 4 g sample aliquots were prepared. The samples were spiked with deuterated internal standards and extracted three times by ultra- sonicator for a 15-min period each with 35 ml of DCM in a cool water bath (10- 15 °C). The sonication method has been used and recommended by various authors (Trapido, 1999; Agarwal *et al.*, 2006). Filtered extracts were shaken overnight with activated copper to eliminate sulfur. The extracts were then concentrated to about 2 ml by rotary evaporator and adjusted exactly to 2 ml by first, letting all the solvent evaporated and second, adding 2ml of DCM to the extract. 500 µl of the extracts were used for a fractionation step, while another 1 ml transferred to a pre- weighed vial and left to dry before determining the total extracted lipid (TEL) yield.

3.2.6 Sulfur removal

As described in Chapter 2 (Section 2.2.6).

3.2.7 Fractionation

As described in Chapter 2 (Section 2.2.7.2).

3.2.8 Instrumental analysis

As described in Chapter 2 (Section 2.2.8).

3.2.9 Quality assurance

Reproducibility, procedural blanks and instrument detection limit (IDL) analysis are as per described in Chapter 2 (Section 2.2.9).

3.2.9.1 Method detection limit (MDL)

The procedure is as per described in Section 2.2.9.3.2. Results of the analysis are shown in Table 3.4 below:

Table 3.4 GC- MS Method detection limit (MDL) for this PAHs study

Compounds	Method Detection Limit (mg kg^{-1})	
	Road Dusts	**Road Side Soils**
Naphthalene	0.36	0.31
Acenaphthylene	0.11	0.06
Acenaphthene	0.16	0.18
Fluorene	0.34	0.32
Phenanthrene	0.37	0.22
Anthracene	0.22	0.25
Fluoranthene	0.15	0.12
Pyrene	0.14	0.16
Benz[a]anthracene	0.18	0.13
Chrysene	0.22	0.28
Benzo[b]fluoranthene	0.13	0.18
Benzo[k]fluoranthene	0.07	0.10
Benzo[a]pyrene	0.04	0.06
Indeno[1,2,3- cd]pyrene	0.17	0.20
Dibenz[a,h]anthracene	0.06	0.09
Benzo[g,h,i]perylene	0.13	0.17
Naphthalene- d_8	0.33	0.26
Acenaphtene- d_{10}	0.37	0.23
Phenanthrene- d_{10}	0.30	0.24
Chrysene- d_{12}	0.18	0.15
Perylene- d_{12}	0.35	0.24

3.2.9.2 Recovery studies

The procedures are as per described in Section 2.2.9.4. The result for the multi- step recoveries is shown in Table 3.5:

Table 3.5 The results of multi- step recoveries including the surrogates' recoveries in percentage (%) for road side soils and road dusts

Compounds	% Recovery ($\bar{x}$ ± R.S.D, n=3)	
	Road Side Soils	**Road Dusts**
Naphthalene	28.3 ± 12.6	29.3 ± 13.6
Acenaphthylene	59.7 ± 21.3	64.2 ± 11.6
Acenaphthene	60.8 ± 2.5	54.1 ± 9.1
Fluorene	64.9 ± 7.9	33.8 ± 3.6
Phenanthrene	79.9 ± 23.4	80.9 ± 13.4
Anthracene	72.8 ± 25.9	78.3 ± 6.8
Fluoranthene	32.3 ± 17.0	67.4 ± 15.6
Pyrene	83.2 ± 11.6	79.9 ± 21.3
Benz[a]anthracene	78.1 ± 14.6	76.8 ± 10.1
Chrysene	73.3 ± 7.8	80.0 ± 5.6
Benzo[b]fluoranthene	84.1 ± 2.4	88.4 ± 12.4
Benzo[k]fluoranthene	89.9 ± 12.4	72.1 ± 11.1
Benzo[a]pyrene	84.2 ± 22.3	72.8 ± 11.2
Indeno[1,2,3- cd]pyrene	65.4 ± 17.8	51.3 ± 22.4
Dibenz[a,h]anthracene	77.3 ± 10.1	73.2 ± 11.1
Benzo[g,h,i]perylene	83.0 ± 22.6	80.3 ± 13.2
Acenaphtene- d_{10}	63.9 ± 20.8	80.8 ± 25.1
Phenanthrene- d_{10}	76.1 ± 21.9	88.6 ± 20.4
Chrysene- d_{12}	60.0 ± 10.5	89.5 ± 24.1
Perylene- d_{12}	76.2 ± 17.0	79.3 ± 27.1

Industrial soil Certified Reference Material (CRM) No 524 (Sample Identification No.: 309) from the European Commission, Community Bureau of Reference was also analyzed as CRM in Chapter 2 to assess the accuracy and precision of methods. The results were in good agreement with the certified values (Table 3.6).

Table 3.6 The results of multi- step recoveries by Certified Reference Materials (CRM) No 524: Industrial soil

Compound	Industrial Soil (CRM-524)		
	Certified Conc. ($\mu g\ g^{-1}$)	Found Conc. ($\mu g\ g^{-1}$)	% Error
Pyrene	173 ± 11	181 ± 13	4.62
Benz[a]anthracene	22.5 ± 1.8	21.3 ± 2.1	5.33
Benzo[a]pyrene	8.6 ± 0.5	7.12 ± 1.6	17.2
Benzo[e]pyrene	10.6 ± 1.4	12.4 ± 2.3	17.0
Benzo[b]fluoranthene	13.5 ± 1.6	12.6 ± 1.1	6.67
Benzo[k]fluoranthene	6.2 ± 0.7	6.51 ± 0.5	5.01
Indeno[1,2,3- cd]pyrene	5.1 ± 0.4	4.95 ± 1.1	2.94

3.3 Results and discussion

3.3.1 PAH distributions in road side soils

3.3.1.1 Industrial areas

The summary of PAH concentrations and means in road side soils of the industrial areas are given in Table 3.7. The Chan Saw Lin, Segambut, Kepong- 1 and Kepong- 2 areas were each represented by 6 sampling sites, while the Kuchai area by 7 sites. The highest total PAHs concentration detected was in the Chan Saw Lin area with 18384 ± 12324 $\mu g\ kg^{-1}$, while the lowest was in the Kepong- 2 area with 2804 ± 2645 $\mu g\ kg^{-1}$. Reports on PAH concentrations limits are sparse worldwide, and in Malaysia there are still no standard limits.

It is known that besides industrial sources, PAHs are contributed extensively by vehicle emissions (Nelson *et al.*, 2008). Among these industrial areas, Chan Saw Lin, Segambut and Kuchai do have higher traffic densities compared to Kepong- 1 and Kepong- 2. Thus the PAHs in the Chan Saw Lin area might also be from traffic on the adjacent Tun Razak Street, a major road skirting the western part of the inner city.

In terms of individual PAHs, Indeno[1,2,3- cd]pyrene was most abundant in Chan Saw Lin, Segambut, and Kepong- 1, at 4616, 1527 and 751 $\mu g\ kg^{-1}$ respectively. In

contrast, phenanthrene was the most abundant PAH in soils from Kuchai at 1336 µg kg^{-1}, and Kepong- 2 had pyrene as most abundant at 448 µg kg^{-1}. Even though the soils of Kuchai and Kepong- 2 were not dominated by the same PAH as the other locales, indeno[1,2,3- cd]pyrene was the second most abundant PAH. According to Dallarosa *et al.* (2008) and Guo *et al.* (2003), the abundance of indeno[1,2,3- cd]pyrene indicates that there is a strong contribution from traffic emissions. On the whole, more than half of the individual PAHs in the soils were higher than the 100 µg kg^{-1} limit set by Polish regulations (Maliszewska-Kordybach *et al.*, 2009).

Table 3.7 Concentrations of PAH in road side soils of industrial areas around Kuala Lumpur city

COMPOUND	CHAN SAW LIN (µg kg^{-1} dw, n= 6)			KUCHAI (µg kg^{-1} dw, n= 7)			SEGAMBUT (µg kg^{-1} dw, n= 6)		
	Mean ± S.D.	**Min**	**Max**	**Mean ± S.D.**	**Min**	**Max**	**Mean ± S.D.**	**Min**	**Max**
Acy	1867.1 ± 1258.4	329.3	3317.1	62.9 ± 51.8	nd	128.1	678.1 ± 226.2	315.2	947.2
Ace	25.3 ± 29.2	nd	64.5	7.3 ± 7.0	nd	15.5	63.4 ± 22.4	39.9	101.1
Flu	80.2 ± 57.3	31.9	166.0	48.8 ± 55.4	nd	131.4	111.3 ± 68.4	46.1	197.4
Phen	707.1 ± 400.3	114.2	1287.4	1336.1 ± 1946.4	70.4	5519.2	592.6 ± 187.0	330.3	806.7
Ant	281.0 ± 267.1	0.0	600.2	124.2 ± 87.8	6.6	288.9	224.3 ± 164.9	65.4	520.3
Flt	1250.9 ± 1322.1	102.1	3829.1	554.2 ± 647.2	62.6	1951.5	263.2 ± 149.5	77.0	446.0
Pyr	2401.6 ± 2460.0	374.3	6926.4	343.1 ± 278.1	36.3	875.3	464.5 ± 321.3	118.2	889.7
BaA	613.1 ± 826.2	113.8	2264.4	123.5 ± 126.4	10.8	343.6	143.9 ± 118.6	35.3	304.4
Chrys	2248.2 ± 1529.5	390.1	4546.8	352.8 ± 300.8	63.7	827.7	523.0 ± 418.0	140.1	1125.5
BbF	1733.4 ± 1856.2	145.6	5264.2	397.3 ± 405.9	36.9	1064.2	431.7 ± 367.7	121.3	1050.5
BkF	1007.7 ± 643.9	131.3	1686.3	166.2 ± 136.6	17.1	312.3	277.0 ± 369.1	44.4	1019.3
BaP	690.4 ± 788.6	54.0	2232.5	114.2 ± 103.7	9.4	280.6	174.5 ± 183.4	28.8	516.2
DbA	627.1 ± 595.2	79.2	1704.1	87.2 ± 79.8	nd	180.1	179.1 ± 220.3	nd	570.3
BgP	230.9 ± 281.1	17.8	752.0	31.1 ± 27.5	nd	65.2	35.1 ± 57.7	nd	135.6
InP	4616.2 ± 3460.5	1098.2	9116.5	888.6 ± 959.3	40.6	2314.3	1527.3 ± 1327.4	146.2	3801.2
Total ± S.D.	18384.1 ± 12324.2			4639.0 ± 3012.2			5690.1 ± 3938.4		

nd: not detected

COMPOUND	KEPONG- 1 (µg kg^{-1} dw, n= 6)			KEPONG- 2 (µg kg^{-1} dw, n= 6)			
	Mean ± S.D.	**Min**	**Max**	**Mean ± S.D.**	**Min**	**Max**	**Total ± S.D.**
Acy	151.2 ± 40.3	104.2	202.4	141.2 ± 34.7	110.6	202.1	2909.0 ± 760.1
Ace	17.8 ± 15.2	nd	36.7	30.3 ± 8.8	23.0	45.1	144.0 ± 21.2
Flu	59.4 ± 41.6	10.6	114.1	68.1 ± 35.8	36.0	123.5	368.3 ± 24.2
Phen	383.2 ± 208.3	159.2	659.0	393.5 ± 228.7	191.4	726.2	3413.9 ± 390.1
Ant	114.3 ± 85.6	32.1	241.5	80.8 ± 57.2	35.5	183.9	825.5 ± 84.3
Flt	295.3 ± 246.1	64.9	606.4	299.5 ± 385.3	43.3	1056.1	2663.7 ± 418.0
Pyr	374.4 ± 301.8	59.0	805.6	448.4 ± 509.2	47.1	1424.0	4033.1 ± 893.1
BaA	118.4 ± 103.2	7.8	268.3	97.4 ± 149.6	20.2	402.4	1096.7 ± 221.1
Chrys	364.3 ± 272.1	23.6	645.0	333.6 ± 362.3	39.6	957.3	3822.6 ± 832.9
BbF	303.8 ± 286.9	27.2	731.1	265.5 ± 422.2	38.9	1124.7	3132.3 ± 622.7
BkF	151.1 ± 131.0	11.7	312.4	80.9 ± 112.1	9.2	306.1	1684.6 ± 381.6
BaP	100.6 ± 100.5	nd	257.5	80.4 ± 130.9	1.8	345.0	1160.9 ± 258.9
DbA	85.9 ± 76.5	nd	167.1	40.8 ± 61.6	nd	158.4	1020.5 ± 241.9
BgP	18.5 ± 20.3	nd	39.1	nd	nd	nd	315.3 ± 94.6
InP	751.3 ± 664.4	73.3	1760.7	444.4 ± 391.2	26.2	935.2	8228.2 ± 1707.1
Total ± S.D.	3292.0 ± 2366.4			2804.0 ± 2645.3			34811.1 ± 6485.0

nd: not detected

3.3.1.2 Commercial areas

As for the commercial areas, the mean, standard deviation, minimum and maximum concentrations of PAHs at each sampling sites are shown in Table 3.8. Commercial areas of Ampang Park, Sultan Ismail and Masjid India were represented by 6 samples in each area while Raja Chulan, Bukit Bintang and Imbi were represented by 5, 4, and 3 samples, respectively. PAHs total concentrations in the studied commercial areas ranged from 25979 ± 39357 µg kg^{-1} (Imbi) to 333039 ± 623111 µg kg^{-1} (Ampang Park). These result shows that commercial areas of Kuala Lumpur city were much more contaminated by PAHs than the industrial areas. This is probably due to greater number of motor vehicles plying the commercial areas everyday compared to industrial areas. Sampling site characteristics with high density of population and various community activities might also affected the results as identified by Fadzil *et al.* (2008).

Maliszewka- Kordybach (1996) had described that soil with total PAHs greater than 1000 µg kg^{-1} as heavily contaminated while total concentration in between 600 to 1000 µg kg^{-1} as contaminated and total concentration less than 200 µg kg^{-1} were described as not contaminated. Comparing total concentrations found in commercial areas as well as in industrial areas of this study, it can be said that all sampling sites has been heavily contaminated with PAHs.

For the individual PAH compound in commercial areas, it was found that indeno[1,2,3- cd]pyrene was the greatest contributor of PAHs at all sampling sites with 102660 ± 139417 µg kg^{-1} in Ampang Park, 63845 ± 26304 µg kg^{-1} in Sultan Ismail, 51543 ± 45693 µg kg^{-1} in Masjid India, 25436 ± 30945 µg kg^{-1} in Bukit Bintang, 42889 ± 21768 µg kg^{-1} in Imbi and 46353 ± 21720 µg kg^{-1} in Raja Chulan. According to USEPA (1994), there are still no record on the impact of indeno[1,2,3- cd]pyrene towards human while data

on the impact of this compound in animal bioassays are still not sufficient. However, among the 16 listed PAHs by USEPA, it still listed as human carcinogens along with 6 others individual PAHs namely benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene and dibenz[a,h]anthracene while remaining 9 other compounds are listed as non- carcinogens (USEPA, 2002). Indeno[1,2,3- cd]pyrene has also been found to cause tumors in mice and positive in bacterial gene mutation assays. Acenaphtene and fluorene has not been detected at all sampling sites indicating lower effect of coal tar on the samples.

Table 3.8 Concentrations of PAH in road side soils of commercial areas around Kuala Lumpur city

COMPOUND	AMPANG PARK (μg kg^{-1} dw, n= 6)			SULTAN ISMAIL (μg kg^{-1} dw, n= 6)		
	Mean ± S.D.	**Min**	**Max**	**Mean ± S.D.**	**Min**	**Max**
Acy	2711.1 ± 7542.3	nd	18842.2	771.1 ± 369.4	195.2	1812.3
Ace	85.9 ± 83.5	nd	204.3	91.0 ± 120.3	nd	303.1
Flu	57.5 ± 50.8	nd	171.4	62.0 ± 33.2	nd	175.2
Phen	1909.4 ± 2474.1	153.3	6482.3	1548.3 ± 539.5	405.1	2537.4
Ant	1524.9 ± 4032.4	22.2	10150.6	456.4 ± 185.7	176.6	989.1
Flt	10581.4 ± 28831.3	152.1	71963.4	3663.2 ± 1828.8	1437.2	8788.0
Pyr	10895.2 ± 26941.7	414.2	67877.9	4800.9 ± 1300.1	2347.7	11578.9
BaA	10692.5 ± 28007.6	590.8	70292.0	3063.2 ± 1550.3	1104.0	6100.3
Chrys	23881.1 ± 52486.3	954.9	133309.1	9610.1 ± 2202.5	3556.9	24834.0
BbF	48651.9 ± 129771.1	927.5	324649.3	10952.3 ± 6590.0	1588.5	22131.2
BkF	5649.5 ± 6974.2	264.3	16929.1	3055.3 ± 1886.2	nd	5625.9
BaP	30722.7 ± 75730.5	nd	188584.9	8475.2 ± 2906.6	811.3	30921.0
DbA	43901.9 ± 105191.0	1253.1	265754.0	13079.3 ± 6065.3	3602.1	26953.3
BgP	9256.3 ± 19899.2	nd	50222.3	3001.1 ± 1238.2	nd	10126.1
InP	102660.5 ± 139417.7	1011.0	368016.2	63845.2 ± 26304.4	15861.2	154257.4
Total ± S.D.	333039.2 ± 623111.4			86322.1 ± 40534.0		

nd: not detected

COMPOUND	MASJID INDIA (µg kg^{-1} dw, n= 6)			BUKIT BINTANG (µg kg^{-1} dw, n= 4)		
	Mean ± S.D.	**Min**	**Max**	**Mean ± S.D.**	**Min**	**Max**
Acy	1078.1 ± 633.2	171.1	2412.1	435.1 ± 300.1	87.3	759.1
Ace	109.3 ± 124.3	nd	330.3	42.3 ± 45.4	nd	109.4
Flu	83.9 ± 48.6	nd	203.2	43.5 ± 21.7	nd	91.7
Phen	1562.4 ± 678.2	797.7	2870.0	1031.2 ± 753.1	424.2	2034.3
Ant	612.2 ± 267.2	125.8	1328.9	287.0 ± 182.3	74.8	513.2
Flt	3282.2 ± 1433.0	1144.1	8333.5	1843.4 ± 1345.6	514.1	3601.0
Pyr	4177.5 ± 2249.2	1232.5	10578.3	2642.4 ± 1805.1	687.0	4899.9
BaA	2792.1 ± 2224.9	1048.3	6790.0	1287.7 ± 901.0	300.3	2806.3
Chrys	10581.1 ± 4730.3	3199.6	22132.4	4192.0 ± 2865.5	383.5	8388.5
BbF	11639.3 ± 16752.0	456.6	47201.3	4788.4 ± 4584.4	1572.0	11979.3
BkF	2489.4 ± 872.9	nd	3831.1	2068.3 ± 1962.1	nd	5577.5
BaP	10253.2 ± 9829.1	1233.4	26816.0	3720.0 ± 2923.1	712.4	8318.3
DbA	16209.1 ± 19391.4	4981.0	55024.5	5318.0 ± 5212.4	901.3	13843.0
BgP	2974.5 ± 3562.0	nd	9288.5	895.5 ± 919.2	nd	2698.6
InP	51543.9 ± 45693.1	3589.9	133997.6	25436.4 ± 30945.5	1244.1	81154.5
Total ± S.D.	103244.3 ± 105976.4			44041.0 ± 53902.3		

nd: not detected

COMPOUND	IMBI (µg kg^{-1} dw, n= 3)			RAJA CHULAN (µg kg^{-1} dw, n= 5)			Total ± S.D.		
	Mean ± S.D.	Min	Max	Mean ± S.D.	Min	Max			
Acy	466.1 ± 668.3	nd	1210.1	1027.3 ± 876.2	242.1	2179.5	6490.3	±	843.9
Ace	42.5 ± 52.7	nd	121.4	90.3 ± 103.1	nd	208.6	461.4	±	27.9
Flu	89.6 ± 43.8	nd	307.3	70.8 ± 41.7	nd	135.3	407.1	±	17.1
Phen	1656.6 ± 1218.0	423.1	2859.0	1632.1 ± 1133.0	429.0	2993.0	9338.0	±	289.5
Ant	345.3 ± 349.2	92.7	730.4	513.4 ± 458.4	76.8	1008.9	3738.8	±	457.4
Flt	2538.1 ± 1882.2	1126.2	4750.9	4062.0 ± 3424.9	542.2	7583.3	25974.6	±	3166.9
Pyr	3296.6 ± 1793.3	1133.0	5328.4	4610.4 ± 2928.8	786.3	8707.2	30419.7	±	2968.1
BaA	1517.1 ± 945.1	854.4	2624.1	2918.6 ± 2712.1	464.9	6401.3	22270.3	±	3502.5
Chrys	5464.2 ± 1671.2	2035.9	12079.9	8329.0 ± 4789.4	1302.0	18705.5	62056.5	±	7065.4
BbF	5438.4 ± 3925.3	2118.1	10640.0	5758.4 ± 3454.7	272.8	14802.9	87227.9	±	16970.8
BkF	1446.5 ± 1630.2	486.3	3809.5	3854.4 ± 2925.3	nd	8506.0	18561.5	±	1499.7
BaP	5575.3 ± 2498.2	990.4	14598.8	5560.2 ± 4200.1	597.4	10922.6	64304.7	±	10074.5
DbA	6618.3 ± 4221.0	1749.0	18358.4	9185.0 ± 6311.5	1030.4	20166.5	94310.5	±	14388.1
BgP	1458.1 ± 713.0	nd	5706.3	2783.4 ± 1288.5	nd	10531.4	20366.4	±	3002.0
InP	42889.5 ± 21768.2	8201.3	121320.4	46353.4 ± 21720.1	7395.4	127159.0	332726.6	±	26290.5
Total ± S.D.	25979.3 ± 39357.2			63553.2 ± 57311.1			656178.2	±	113064.4

nd: not detected

3.3.1.3 Residential areas

Concentrations of PAHs in residential areas show a really clear impact of local activities towards PAHs level. Residential areas located adjacent to commercial areas have recorded the highest concentrations of PAHs with 49699 ± 45760 µg kg^{-1} in U- Thant areas and 44383 ± 67347 µg kg^{-1} in Kg. Baru (Table 3.9). Meanwhile, residential areas that are located further from both industrial and commercial areas (≥ 5 km) show the lowest level of PAHs with 1580 ± 2010 µg kg^{-1} in Puncak Jalil and 4973 ± 5164 µg kg^{-1} in Jalan Datuk Sulaiman, TTDI. On the other hand, residential areas located adjacent to industrial areas recorded PAH levels lower than those adjacent to commercial areas and higher than those further away from both commercial and industrial areas with 30857 ± 45445 µg kg^{-1} in Segambut and 30051 ± 23796 µg kg^{-1} in PPR Sg. Besi.

Residential areas of U- Thant and Kg. Baru was found to have lower concentrations of PAHs in comparison to the adjacent commercial area (Ampang Park) with differences ratio of about 1: 7 for U- Thant and 1: 8 for Kg. Baru. These results are different than recorded for residential areas adjacent to industrial areas. Segambut residential area has been recorded to have about five times greater PAH concentrations than Segambut industrial area while PPR Sg. Besi has about two times greater PAH concentrations in comparison to Chan Saw Lin industrial area which is located adjacent to it. The differences between what had happen in residential areas adjacent to commercial areas and residential areas adjacent to industrial areas suggesting that studied areas are affected by various surrounding contributors and might also be subjected to other factors such as type of soils, wind movement, and other indirect activities such as bioformation (Thiele and Brümmer, 2002).

The total concentrations of PAHs recorded in all the studied residential areas can be said as heavily contaminated (Maliszewska- Kordybach, 1996) with PAHs from anthropogenic activities since the recorded concentrations were so much higher than the normal background concentrations (1 to 10 µg kg^{-1}) which are derived from plant synthesis, forest fires and volcanoes (Edwards, 1983).

With only one fourth of the individual compounds having a concentration of less than 100 µg kg^{-1} (limit in Polish regulations) (Maliszewska- Kordybach, 2009), the greatest contributor of PAHs in all sampling sites of residential areas recorded are still indeno[1,2,3-cd]pyrene with 23568 ± 23608 µg kg^{-1} in U- Thant, 23593 ± 36239 µg kg^{-1} in Kg. Baru, 13401 ± 18259 µg kg^{-1} in Segambut, 11900 ± 11416 µg kg^{-1} in PPR Sg. Besi, 644 ± 1051 µg kg^{-1} in Puncak Jalil and 2596 ± 3409 µg kg^{-1} in Jalan Datuk Sulaiman.

Table 3.9 Concentrations of PAH in road side soils of residential areas around Kuala Lumpur city

	U-THANT (µg kg^{-1} dw, n= 6)			KG. BARU (µg kg^{-1} dw, n= 6)		
COMPOUND	**Mean ± S.D.**	**Min**	**Max**	**Mean ± S.D.**	**Min**	**Max**
Acy	361.3 ± 373.2	nd	1024.4	615.1 ± 917.7	nd	2458.3
Ace	180.1 ± 233.2	nd	583.5	38.6 ± 94.4	nd	231.1
Flu	58.4 ± 98.9	nd	254.1	33.8 ± 44.0	nd	110.0
Phen	1509.9 ± 1943.3	82.6	5382.5	833.2 ± 1069.1	77.2	2980.2
Ant	234.3 ± 298.1	nd	818.0	301.0 ± 482.2	nd	1277.1
Flt	2634.0 ± 3448.9	55.2	9242.4	1230.2 ± 1600.4	57.6	4443.6
Pyr	2127.4 ± 2509.8	74.4	6960.6	1300.1 ± 1648.9	64.3	4577.4
BaA	1146.1 ± 1137.2	126.3	3077.7	840.5 ± 969.9	100.1	2774.3
Chrys	3551.3 ± 4065.3	nd	11292.1	2901.3 ± 4053.4	nd	11044.8
BbF	5050.4 ± 6351.2	nd	17427.3	4079.4 ± 6164.5	nd	16500.3
BkF	1990.1 ± 2327.0	24.1	6505.5	1841.0 ± 2870.1	nd	7625.6
BaP	2142.4 ± 2656.4	462.1	7413.3	1835.4 ± 3096.5	nd	8106.1
DbA	4474.7 ± 5708.0	nd	15716.5	4315.1 ± 7164.4	nd	18823.8
BgP	674.4 ± 910.9	nd	2141.3	628.5 ± 1103.6	nd	2811.3
InP	23568.1 ± 23608.3	417.4	65900.6	23593.4 ± 36239.1	nd	96037.7
Total ± S.D.	49699.2 ± 45760.1			44383.1 ± 67347.3		

nd: not detected

COMPOUND	SEGAMBUT (µg kg^{-1} dw, n= 6)			PPR Sg. Besi (µg kg^{-1} dw, n= 6)		
	Mean ± S.D.	**Min**	**Max**	**Mean ± S.D.**	**Min**	**Max**
Acy	270.1 ± 436.3	nd	1152.1	267.1 ± 181.1	nd	486.5
Ace	63.2 ± 98.0	nd	191.4	42.6 ± 49.6	nd	115.3
Flu	24.0 ± 37.4	nd	78.5	31.7 ± 77.7	nd	190.6
Phen	627.7 ± 376.1	120.2	1283.4	647.3 ± 601.3	315.3	1868.3
Ant	189.0 ± 236.5	24.8	652.6	170.1 ± 158.5	60.6	467.5
Flt	923.3 ± 761.3	379.4	2375.3	2306.5 ± 3654.5	341.4	9677.1
Pyr	968.3 ± 873.5	420.1	2700.1	1813.4 ± 2240.6	369.6	6090.0
BaA	983.4 ± 1403.3	296.7	3839.4	1435.1 ± 2086.5	267.4	5616.5
Chrys	2514.5 ± 3401.4	583.5	9396.5	3682.0 ± 5094.7	696.7	13772.4
BbF	3674.4 ± 7125.3	312.3	18191.6	2567.9 ± 2738.2	198.4	7709.6
BkF	1242.4 ± 1894.6	236.0	5089.6	1191.5 ± 971.1	321.3	2521.1
BaP	1722.4 ± 3409.5	160.1	8670.9	1226.5 ± 1103.3	231.9	2759.0
DbA	3790.4 ± 6406.6	296.4	16773.4	2365.1± 1780.7	271.2	4417.9
BgP	468.5 ± 1049.1	nd	2603.1	407.0 ± 457.7	nd	1224.6
InP	13401.5 ± 18259.0	1946.5	49943.0	11900.1 ± 11416.5	4402.1	34440.4
Total ± S.D.	30857.3 ± 45445.4			30051.3 ± 23796.2		

nd: not detected

COMPOUND	PUNCAK JALIL (µg kg^{-1} dw, n= 6)			JLN DATUK SULAIMAN, TTDI (µg kg^{-1} dw, n= 7)					
	Mean ± S.D.	**Min**	**Max**	**Mean ± S.D.**	**Min**	**Max**	**Total ± S.D.**		
Acy	38.8 ± 73.2	nd	182.1	65.3 ± 72.1	nd	180.1	1627.5	±	1253.3
Ace	27.8 ± 68.1	nd	167.3	33.4 ± 88.3	nd	234.4	392.6	±	345.6
Flu	12.7 ± 30.1	nd	74.1	20.5 ± 54.3	nd	144.2	185.8	±	92.7
Phen	127.8 ± 175.1	nd	450.6	240.1 ± 143.3	85.1	507.4	4024.1	±	2915.8
Ant	11.6 ± 18.9	nd	44.8	40.1 ± 52.6	nd	136.6	953.0	±	664.1
Flt	91.0 ± 115.1	nd	303.3	210.7 ± 145.4	27.9	412.8	7429.5	±	6286.0
Pyr	101.2 ± 90.1	nd	254.5	237.4 ± 173.2	35.0	540.1	6584.6	±	4873.5
BaA	187.4 ± 199.4	42.5	581.1	145.1 ± 54.1	44.8	222.0	4760.4	±	3098.6
Chrys	111.4 ± 212.3	nd	534.5	398.4 ± 410.3	64.7	1281.5	13223.5	±	9290.1
BbF	135.5 ± 227.1	nd	574.9	245.5 ± 337.7	nd	946.7	15790.6	±	12229.5
BkF	72.8 ± 105.7	nd	232.0	157.1 ± 183.4	5.0	557.4	6520.1	±	4849.6
BaP	14.6 ± 34.2	nd	84.3	25.9 ± 68.6	nd	182.3	6970.5	±	5583.4
DbA	5.5 ± 13.4	nd	32.7	226.2 ± 394.5	nd	929.7	15213.7	±	12027.6
BgP	nd	nd	nd	336.1 ± 528.5	nd	1334.3	2569.5	±	1438.7
InP	644.5 ± 1051.6	nd	2454.1	2596.1 ± 3409.3	nd	9613.7	76135.6	±	58590.2
Total ± S.D.	1580.2 ± 2010.1			4973.3 ± 5164.0			109363.1	±	113064.3

nd: not detected

3.3.2 PAHs distributions in road dusts

3.3.2.1 Industrial areas

The PAH concentrations in the road dusts of the studied industrial areas ranged from 13581 ± 5295 µg kg^{-1} to 3056 ± 1929 µg kg^{-1} (Table 3.10). The highest concentration was recorded in Segambut which was different from the highest PAHs recorded in road side soils at Chan Saw Lin. This might be due to (1) the PAH source types, e.g. weathered materials, automobile exhaust, lubricating oils, fuels, tire debris, construction material, atmospheric fallout, are more important inputs to Segambut soils, or (2) the Segambut area received more allochthonous road dusts than the other regions by wind and/ or rain runoff transport from one place to another (William *et al.*, 2003; Lee and Dong, 2010). Runoff processes can remove road dust with PAHs into water reservoirs during rainfall (Aryal *et al.*, 2006; Boonyatumanond *et al.*, 2006; Hoffmann *et al.*, 1984). However, the volatile PAHs in road dusts are subject to evaporation into the atmosphere (Manoli *et al.*, 2004; Aryal *et al.*, 2005).

Indeno[1,2,3- cd]pyrene was the major PAH in the road dusts of Chan Saw Lin and Kuchai with 2296 µg kg^{-1} and 1238 µg kg^{-1}, respectively. However, the road dusts of Segambut had acenaphthalene highest with 5331 µg kg^{-1}, while Kepong- 1 and Kepong- 2 had phenanthrene and pyrene highest at 592 µg kg^{-1} and 612 µg kg^{-1}, respectively.

Table 3.10 Concentrations of PAH in road dusts of industrial areas around Kuala Lumpur city

	CHAN SAW LIN (µg kg^{-1} dw, n= 6)			KUCHAI (µg kg^{-1} dw, n= 7)			SEGAMBUT (µg kg^{-1} dw, n= 6)		
COMPOUND	**Mean ± S.D.**	**Min**	**Max**	**Mean ± S.D.**	**Min**	**Max**	**Mean ± S.D.**	**Min**	**Max**
Acy	1092.1 ± 1059.5	358.2	3155.3	122.3 ± 102.6	47.3	273.3	5331.4 ± 3236.5	2818.5	10681.4
Ace	62.2 ± 33.0	19.4	111.5	65.6 ± 45.0	33.2	151.5	765.2 ± 451.4	394.0	1532.3
Flu	62.0 ± 36.7	26.1	118.1	73.4 ± 58.7	28.2	170.2	527.5 ± 286.1	280.4	1014.5
Phen	652.5 ± 569.5	221.4	1732.4	773.2 ± 516.6	299.4	1461.0	1251.4 ± 302.1	758.6	1625.6
Ant	218.1 ± 241.0	58.2	691.9	159.6 ± 128.5	52.7	391.7	539.1 ± 212.5	295.5	848.1
Flt	883.5 ± 1031.6	193.8	2913.0	521.4 ± 354.5	170.7	1234.5	644.5 ± 233.6	246.3	875.3
Pyr	1124.5 ± 1437.1	206.0	3994.6	719.2 ± 591.6	155.9	1954.4	916.5 ± 327.5	335.4	1306.2
BaA	244.1 ± 294.6	31.9	818.8	137.5 ± 130.4	28.8	418.1	175.1 ± 94.0	53.5	324.8
Chrys	1490.7 ± 1457.2	345.4	4265.7	906.6 ± 774.4	273.3	2530.0	1046.6 ± 485.3	382.2	1809.9
BbF	700.4 ± 751.7	137.6	2184.5	372.0 ± 358.9	90.2	1126.5	467.6 ± 200.2	214.5	729.0
BkF	504.6 ± 572.1	70.0	1638.4	279.5 ± 238.3	88.1	751.3	254.5 ± 103.2	157.6	427.7
BaP	592.0 ± 939.9	98.4	2478.7	124.5 ± 126.5	26.8	400.6	151.8 ± 81.7	61.4	270.6
DbA	190.5 ± 209.1	38.7	606.4	80.5 ± 80.3	18.0	234.6	113 ± 79.0	41.6	231.8
BgP	59.5 ± 97.2	nd	253.6	40.9 ± 45.4	nd	116.3	35.5 ± 22.6	17.0	77.6
InP	2296.3 ± 2969.5	282.4	8242.9	1238.3 ± 1154.6	265.6	3412.5	1360.5 ± 904.6	524.1	2787.3
Total ± S.D.	10174.1 ± 11596.0			5615.2 ± 4396.2			13581.1 ± 5295.4		

nd: not detected

COMPOUND	KEPONG- 1 (µg kg^{-1} dw, n= 6) Mean ± S.D.	Min	Max	KEPONG- 2 (µg kg^{-1} dw, n= 6) Mean ± S.D.	Min	Max	Total ± S.D.
Acy	303.3 ± 190.1	154.6	668.8	135.6 ± 52.9	93.2	238.1	6985.6 ± 2235.5
Ace	30.2 ± 24.2	nd	70.8	32.3 ± 7.6	21.7	42.5	955.6 ± 321.4
Flu	57.6 ± 36.3	9.3	107.7	74.5 ± 27.9	47.7	124.4	794.7 ± 206.0
Phen	592.1 ± 392.4	325.5	1364.4	478.7 ± 321.1	256.3	1114.1	3749.4 ± 300.0
Ant	158.8 ± 133.9	67.4	424.9	81.4 ± 56.6	37.8	190.1	1156.9 ± 179.2
Flt	481.7 ± 404.5	228.3	1292.3	330.5 ± 244.6	108.9	779.0	2861.1 ± 206.7
Pyr	536.5 ± 449.1	191.6	1405.2	612.1 ± 378.6	240.1	1314.5	3909.5 ± 239.2
BaA	114.6 ± 125.7	29.3	356.8	104.5 ± 87.3	22.0	227.3	776.8 ± 56.9
Chrys	552.3 ± 525.6	24.1	1541.5	452.5 ± 307.0	20.6	893.2	4447.7 ± 415.6
BbF	297.7 ± 305.6	118.2	916.9	192.5 ±160.6	67.3	428.6	2030.1 ± 192.8
BkF	133.7 ± 117.5	41.4	349.4	88.2 ± 94.7	8.1	238.5	1260.8 ± 162.4
BaP	71.5 ± 75.9	19.1	223.6	85.6 ± 91.1	15.5	265.2	1026.0 ± 218.7
DbA	39.3 ± 36.7	14.3	109.7	37.8 ± 54.8	nd	147.3	461.5 ± 63.3
BgP	13.1 ± 21.3	nd	49.5	5.5 ± 10.7	nd	26.7	154.4 ± 21.8
InP	396.6 ± 303.0	87.7	883.3	344.7 ± 262.5	93.4	840.1	5636.2 ± 803.3
Total ± S.D.	3777.1 ± 2968.2			3056.2 ± 1929.0			36206.3 ± 4498.1

nd: not detected

3.3.2.2 Commercial areas

The total PAHs in road dusts of the studied commercial areas was recorded highest in Ampang Park with 329299 ± 166569 µg kg^{-1} followed by Sultan Ismail with 305366 ± 103142 µg kg^{-1}, Masjid India with 208183 ± 68923 µg kg^{-1}, Raja Chulan with 156044 ± 215161 µg kg^{-1}, Imbi with 99381 ± 151580 µg kg^{-1} and Bukit Bintang with 32837 ± 29186 µg kg^{-1} (Table 3.11). These results show that PAH concentrations in road dusts of commercial areas were also greater than in industrial areas which ranging from 3056 ± 1929 µg kg^{-1} (Kepong- 2) to 13581 ± 5292 µg kg^{-1} (Segambut). These findings are in contradiction with what had been found by Wang *et al.* (2011) in Guangzhou, China, Dong and Lee (2009) in Ulsan, Korea and Boonyatumanond *et al.* (2007) in Thailand. The results told that wastes from industrial areas in Kuala Lumpur city are not dominating the contamination level of PAHs in Kuala Lumpur city as in other countries. Dong and Lee (2009) in their study has suggested that emission released by slow moving vehicles and limited dispersion of pollutants in commercial areas can leads to accumulation of high concentrations of PAHs.

On the other hand, indeno[1,2,3- cd]pyrene has also been identified as the most abundance individual PAHs found in road dusts of commercial areas with 190851 ± 106186 µg kg^{-1} in Ampang Park, 154257 ± 49243 µg kg^{-1} in Sultan Ismail, 88450 ± 30435 µg kg^{-1} in Masjid India, 10436 ± 11396 µg kg^{-1} in Bukit Bintang, 121320 ± 126769 µg kg^{-1} in Imbi, and 127159 ± 71234 µg kg^{-1} in Raja Chulan. This compound is one of the indicators of combustion process (Hwang *et al.*, 2003).

Table 3.11 Concentrations of PAH in road dusts of commercial areas around Kuala Lumpur city

	AMPANG PARK (μg kg^{-1} dw, n= 6)			SULTAN ISMAIL(μg kg^{-1} dw, n= 6)		
COMPOUND	**Mean ± S.D.**	**Min**	**Max**	**Mean ± S.D.**	**Min**	**Max**
Acy	623.2 ± 457.4	247.4	1346.4	1812.4 ± 876.6	766.1	3313.3
Ace	119.1 ± 86.2	nd	253.2	174.3 ± 83.0	99.2	330.2
Flu	171.8 ± 285.5	nd	741.5	175.1 ± 124.6	51.1	394.4
Phen	1901.2 ± 681.4	1272.5	3080.3	2537.6 ± 1752.5	1209.3	5990.1
Ant	507.7 ± 326.2	22.2	962.2	989.1 ± 581.6	416.7	1929.7
Flt	4226.5 ± 2251.2	1136.4	6756.1	8788.4 ± 5075.4	5052.9	18799.9
Pyr	6684.4 ± 4124.5	1804.7	12771.5	11579.1 ± 7246.8	6895.5	26190.6
BaA	4228.6 ± 2180.1	2051.6	7313.4	6100.4 ± 2886.3	3148.4	10866.4
Chrys	19820.5 ± 8433.6	9733.6	29749.6	24834.6 ± 7207.2	16255.6	36263.2
BbF	20998.7 ± 11163.5	6006.2	36581.1	22131.5 ± 7293.4	15506.3	35679.6
BkF	8004.1 ± 7864.5	1298.0	21592.7	3991.6 ± 2478.7	nd	7063.8
BaP	29023.3 ± 17952.6	11776.5	52131.9	30921.7 ± 17419.3	8925.2	52539.4
DbA	31015.7 ± 18915.4	8565.3	61218.8	26953.3 ± 12432.6	17648.4	50864.2
BgP	11127.6 ± 5327.5	4229.5	17736.2	10126.1 ± 5804.5	5239.6	20755.0
InP	190851.6 ± 106186.2	49769.6	342796.1	154257.6 ± 49243.3	107265.1	238412.6
Total ± S.D.	329299.1 ± 166569.4			305366.1 ± 103142.0		

nd: not detected

	MASJID INDIA (μg kg^{-1} dw, n= 6)			BUKIT BINTANG (μg kg^{-1} dw, n= 4)		
COMPOUND	**Mean ± S.D.**	**Min**	**Max**	**Mean ± S.D.**	**Min**	**Max**
Acy	2413.5 ± 1089.4	1413.1	4358.6	488.1 ± 319.9	109.3	851.6
Ace	164.1 ± 83.1	96.3	305.2	109.3 ± 87.1	54.2	238.1
Flu	203.5 ± 106.6	81.2	377.5	91.7 ± 47.5	50.4	156.8
Phen	2870.5 ± 2469.0	1262.4	7847.6	1129.6 ± 695.4	655.1	2150.1
Ant	1328.2 ± 650.6	803.3	2332.1	322.2 ± 192.5	103.3	539.6
Flt	8333.4 ± 4685.6	3584.7	17290.0	3069.5 ± 1942.1	1144.1	5578.4
Pyr	10578.2 ± 6356.9	5301.2	22615.2	4896.1 ± 3173.3	1586.3	8008.7
BaA	5825.3 ± 2567.7	2479.9	9607.5	1203.3 ± 1021.5	300.0	2137.2
Chrys	22132.1 ± 5922.3	17211.2	31501.4	6204.2 ± 6708.6	383.7	12648.4
BbF	12595.3 ± 6990.6	456.6	20133.2	4775.6 ± 1701.3	2235.8	5784.5
BkF	2968.1 ± 2070.5	nd	5171.4	1386.2 ± 1222.6	nd	2958.6
BaP	24237.6 ± 6500.3	16568.4	31789.0	8318.9 ± 6509.1	1907.1	13956.7
DbA	20167.6 ± 4933.3	15176.1	27881.8	4130.3 ± 3681.4	907	8990.1
BgP	5920.0 ± 1635.8	4151.4	8445.9	2698.8 ± 3793.2	nd	8272.1
InP	88450.6 ± 30435.7	58854.7	142893.2	10436.0 ± 11396.9	1244.4	26198.4
Total ± S.D.	208183.0 ± 68923.3			32837.3 ± 29186.1		

nd: not detected

COMPOUND	IMBI (µg kg^{-1} dw, n= 3)			RAJA CHULAN (µg kg^{-1} dw, n= 5)			Total ± S.D.		
	Mean ± S.D.	Min	Max	Mean ± S.D.	Min	Max			
Acy	641.9 ± 355.4	366.0	1042.1	685.3 ± 2177.1	242.2	1413.2	6662.2	±	598.3
Ace	121.1 ± 126.6	nd	251.4	72.4 ± 134.6	nd	112.1	760.5	±	126.5
Flu	307.6 ± 418.7	64.9	789.9	124.0 ± 180.9	nd	181.0	1072.1	±	413.2
Phen	2624.4 ± 3043.3	700.8	6132.0	2442.1 ± 3989.4	923.3	3371.3	13503.0	±	2606.0
Ant	478.5 ± 355.1	261.1	887.2	532.1 ± 1140.4	191.3	705.5	4157.5	±	361.9
Flt	3562.3 ± 3143.1	1200.0	7129.5	6474.1 ± 8704.5	2787.5	7993.8	34452.4	±	2790.5
Pyr	5328.1 ± 4938.7	2363.2	11029.2	8707.5 ± 11224.1	4453.3	10187.1	47772.3	±	3579.8
BaA	2105.6 ± 1210.3	854.1	3270.0	4240.7 ± 4868.1	2164.0	6349.0	23701.9	±	2169.0
Chrys	12079.7 ± 7806.6	5183.5	20553.1	18705.6 ± 15576.3	8983.3	29251.4	103774.6	±	10168.4
BbF	10640.2 ± 9152.6	2118.9	20312.4	14802.9 ± 10883.3	272.5	36624.6	85940.8	±	18208.6
BkF	896.9 ± 665.0	486.0	1663.3	8506.6 ± 2985.1	nd	24620.6	25750.0	±	13760.7
BaP	14598.1 ± 7954.5	5414.5	19238.2	10922.3 ± 14942.1	3926.5	24017.0	118018.4	±	10495.8
DbA	18358.6 ± 19326.3	2076.3	39715.0	20166.4 ± 13497.2	5066.6	52431.3	120788.7	±	24515.9
BgP	5706.1 ± 6756.3	347.5	13296.5	10531.0 ± 4223.3	2469.3	31522.9	46108.1	±	14683.1
InP	121320.6 ± 126769.5	18122.6	262825.1	127159.1 ± 71234.7	30345.9	344228.7	692473.6	±	162888.3
Total ± S.D.	99381.4 ± 151580.0			156044.1 ± 215161.3			1131109.0	±	253134.2

nd: not detected

3.3.2.3 Residential areas

Dong and Lee (2009) mentioned that level of total PAHs in road dust of urban areas can be affected by a few characteristics of sampling sites such as location, main type of vehicles in an area and its traffic density. PAH total concentrations of the studied areas: U- Thant (1043245 ± 156396 µg kg^{-1}) > Kg. Baru (344554 ± 204762 µg kg^{-1}) > PPR Sg. Besi (221965 ± 121497 µg kg^{-1}) > Segambut (145726 ± 75812 µg kg^{-1}) > Puncak Jalil (106264 ± 63179 µg kg^{-1}) > Jln Datuk Sulaiman (41984 ± 39623 µg kg^{-1}) (Table 3.12). The results still show that residential areas close to commercial areas (U- Thant and Kg. Baru) had the greatest concentration in comparison to residential areas close to industrial areas (PPR Sg. Besi and Segambut) and residential areas that are far from both industrial and commercial areas (Puncak Jalil and Jln Datuk Sulaiman).

There were significant difference in between level of PAHs in road dusts and road side soils even in residential areas that are far from both commercial and industrial activities. PAHs in road dusts of U- Thant residential area was 20 times greater than the road side soils while road dusts in Kg. Baru were 8 times greater than the road side soils. As for the PPR Sg. Besi and Segambut, road dusts at both locations have 5 and 7 times higher PAH concentrations than the road side soils, respectively. The concentration of PAHs in road dusts of Puncak Jalil is about 67 times higher than the PAHs in road side soils while the concentrations of PAHs in road dusts of Jln. Datuk Sulaiman was 4 times higher than in road side soils. Big difference between total concentrations of PAHs in road dusts and road side soils in all residential areas might be due to the smaller grain size of road dusts in comparison to road side soils as PAH concentrations generally increase with the decreasing particle size (Lau and Stenstrom, 2005). As road dusts are subjected to be moved by the wind, there is also a possibility of road dusts with high PAH levels originated

from other polluted areas to the studied areas, causing the concentrations to become greater than the surrounding soils.

Even though the concentrations recorded were higher than the total PAH concentrations in road side soils, their trend of distribution is still similar, residential areas close to commercial areas are found to record greater PAH levels than the industrial areas and the residential areas that are far from both industrial and commercial areas. The only difference recorded was that the PAH levels in road dusts of the U- Thant residential area were about three times higher in comparison to the nearest commercial areas (Ampang Park) while the concentrations at the Kg. Baru residential area are one time greater than the Ampang Park. On the other hand, road dusts in PPR Sg. Besi show a concentration of PAHs about 22 times higher than the nearby industrial area (Chan Saw Lin) while road dusts in Segambut residential area has about 11 times greater concentration of PAHs than the road dust in adjacent industrial area (Segambut industrial area). Higher concentrations of PAHs in road dusts of residential areas than the adjacent commercial/ industrial areas suggesting that road dusts from the commercial/ industrial areas has been moved by wind or washed by the rain from the other areas to the residential areas. There were also the possibility that the results were affected by road dusts moved from other places and also possible greater traffic volume in residential areas.

On the other hand, for the individual PAHs, indeno[1,2,3- cd]pyrene remains to be the individual PAHs with highest concentrations in all road dusts of the studied residential areas with Kg. Baru (155583 ± 93592 µg kg^{-1}) > PPR Sg. Besi (127519 ± 73055 µg kg^{-1}) > U- Thant (89503 ± 69509 µg kg^{-1}) > Segambut (79107 ± 49719 µg kg^{-1}) > Puncak Jalil (49883 ± 27939 µg kg^{-1}) > Jln. Datuk Sulaiman (20498 ± 21165 µg kg^{-1}).

Table 3.12 Concentrations of PAH in road dusts of residential areas around Kuala Lumpur city

	U- THANT (µg kg^{-1} dw, n= 6)			KG. BARU (µg kg^{-1} dw, n= 6)		
COMPOUND	**Mean ± S.D.**	**Min**	**Max**	**Mean ± S.D.**	**Min**	**Max**
Acy	1183.8 ± 1404.1	344.1	3975.9	1579.5 ± 916.3	598.1	3182.1
Ace	264.4 ± 129.6	84.9	379.6	713.4 ± 208.1	337.4	896.3
Flu	146.6 ± 70.3	53.6	247.3	240.4 ± 77.1	161.3	359.4
Phen	1478.1 ± 1135.3	539.3	3654.4	2878.4 ± 1611.4	1415.2	5568.6
Ant	536.0 ± 710.9	155.3	1973.1	751.1 ± 485.5	332.0	1678.4
Flt	5784.4 ± 8007.1	1446.1	21862.3	9811.4 ± 6933.0	2740.8	21303.9
Pyr	6560.5 ± 8883.5	1204.0	24109.3	9543.5 ± 7151.0	2827.6	21380.1
BaA	4301.1 ± 5507.9	1074.0	15365.5	6499.3 ± 5152.3	1217.7	15415.4
Chrys	17782.2 ± 19555.1	4977.7	56021.6	37497.1 ± 21636.3	11211.1	73755.6
BbF	16949.5 ± 19962.3	3848.3	55747.8	34977.1 ± 22699.3	4533.4	70321.8
BkF	8470.1 ± 7765.4	2454.1	23205.3	17264.4 ± 8244.5	6675.5	29792.1
BaP	8234.4 ± 9388.6	1606.2	26466.4	13838.1 ± 9497.6	4162.7	30384.0
DbA	6738.8 ± 4970.1	2718.2	15271.5	38630.4 ± 22028.4	16725.3	77298.6
BgP	5947.1 ± 8114.5	nd	21949.3	14751.2 ± 8140.4	4389.6	27389.7
InP	89503.1 ± 69509.6	19671.1	201817.3	155583.1 ± 93592.4	58012.9	310424.1
Total ± S.D.	1043245.4 ± 156396.1			344554.2 ± 204762.1		

nd: not detected

	SEGAMBUT (µg kg^{-1} dw, n= 6)			PPR SG. BESI (µg kg^{-1} dw, n= 6)		
COMPOUND	**Mean ± S.D.**	**Min**	**Max**	**Mean ± S.D.**	**Min**	**Max**
Acy	729.1 ± 433.3	254.1	1296.3	429.3 ± 319.4	114.2	838.5
Ace	232.4 ± 200.1	66.0	582.1	204.1 ± 160.5	46.0	444.6
Flu	209.6 ± 87.3	83.3	313.3	177.5 ± 63.4	93.2	257.1
Phen	1450.4 ± 389.0	717.7	1847.2	1266.4 ± 633.5	605.1	2274.0
Ant	370.3 ± 177.5	151.5	573.4	279.6 ± 177.1	78.1	465.5
Flt	3427.1 ± 1111.3	1453.6	4354.1	2658.4 ± 1498.5	904.5	4396.7
Pyr	3623.5 ± 1039.5	1897.0	4661.2	3825.5 ± 2357.2	1094.0	5932.7
BaA	2039.1 ± 1016.4	1210.9	3547.7	2143.4 ± 1370.5	580.7	3618.1
Chrys	13990.2 ± 5488.5	6197.2	20980.5	13303.5 ± 6375.1	7238.3	19350.0
BbF	13485.1 ± 6699.4	5509.4	23942.8	16558.0 ± 8129.0	6738.5	24408.5
BkF	6583.4 ± 3176.1	2402.3	10479.4	11704.3 ± 6352.3	5409.3	20090.4
BaP	4416.3 ± 2317.6	1747.4	8123.9	7840.0 ± 4664.1	3118.0	14227.5
DbA	12612.5 ± 8148.6	911.6	20886.0	23327.7 ± 11629.0	4500.7	34069.3
BgP	3456.1 ± 2818.7	nd	6543.8	10733.9 ± 9665.8	169.4	25274.2
InP	79107.5 ± 49719.3	24792.1	158200.3	127519.4 ± 73055.2	28192.9	227488.6
Total ± S.D.	145726.3 ± 75812.2			221965.1 ± 121497.3		

nd: not detected

COMPOUND	PUNCAK JALIL (µg kg^{-1} dw, n= 6)			JLN DATUK SULAIMAN, TTDI (µg kg^{-1} dw, n= 7)			Total ± S.D.		
	Mean ± S.D.	Min	Max	Mean ± S.D.	Min	Max			
Acy	315.5 ± 331.1	81.3	958.5	147.3 ± 150.1	nd	412.1	4406.6	±	3287.7
Ace	254.3 ± 166.4	78.1	520.6	184.4 ± 158.3	nd	429.4	1881.1	±	1181.4
Flu	89.6 ± 50.8	15.4	149.7	126.6 ± 104.6	0.2	320.1	1009.5	±	318.6
Phen	569.3 ± 233.4	291.2	928.9	685.0 ± 577.1	315.1	1964.0	8441.6	±	4847.5
Ant	150.3 ± 154.4	38.1	455.1	77.9 ± 91.3	23.4	282.4	2175.4	±	1487.3
Flt	981.0 ± 459.0	447.5	1797.1	924.4 ± 790.3	221.3	2576.8	23738.6	±	20209.0
Pyr	1067.9 ± 506.3	398.1	1813.1	1263.5 ± 1121.1	339.2	3640.1	26090.2	±	19294.5
BaA	1316.3 ± 1175.3	523.3	3674.0	1433.3 ± 686.0	797.9	2915.0	17971.6	±	12058.6
Chrys	10846.1 ± 7683.4	3023.3	25633.6	5315.1 ± 4460.1	1205.5	14240.4	99619.9	±	65564.7
BbF	12066.4 ± 10384.0	3046.0	32056.7	2604.2 ± 2121.9	nd	5582.8	97072.4	±	62982.5
BkF	8909.1 ± 5633.4	2321.8	16159.8	2824.5 ± 2533.2	385.6	7583.6	56224.6	±	28582.0
BaP	4606.5 ± 4047.1	1011.6	12208.5	1740.9 ± 1152.4	476.1	3860.5	40965.1	±	24873.4
DbA	8609.4 ± 6539.4	2372.7	19809.6	3020.1 ± 4204.4	nd	11930.3	93438.4	±	79249.5
BgP	6603.0 ± 5759.9	817.3	14443.2	1144.5 ± 1401.1	nd	3865.6	42825.3	±	29373.6
InP	49883.1 ± 27939.3	24630.4	95901.4	20498.9 ± 21165.4	2781.6	64846.5	525510.2	±	291102.3
Total ± S.D.	106264.2 ± 63179.1			41984.4 ± 39623.0			188518.1	±	115841.1

nd: not detected

3.3.3 Comparison between total PAH distributions in road side soils and road dusts of these and other areas

The PAH concentrations reported in soil and road dusts of industrial, commercial and residential areas worldwide and in Malaysia are compared in Table 3.13 and 3.14. The PAH levels in these soils are high compared to the other areas while those in road dust are intermediate for industrial areas and higher for commercial and residential areas. Within Malaysia, comparison shows that the PAHs in the soils were high, but still lower than the greatest value reported for urban Kota Bahru and those in road dust were higher than in urban Kuala Lumpur.

Table 3.13 Comparison between PAHs in road dusts and road side soils of various areas studied

Soil			Road Dust		
Location	**Concentration (µg kg^{-1} dw)**	**No. of PAHs**	**Location**	**Concentration (µg kg^{-1} dw)**	**No. of PAHs**
Industrial Area					
Angren, Uzbekistan (Bandowe *et al.*, 2010)	118- 5913	31	Taichung Industrial Park, Taiwan (Fang *et al.*, 2004)	26700	21
El Paso, Texas, USA (Torre-Roche *et al.*, 2009)	0.1– 2226	16	Industrial site, Lahore, Pakistan (Smith *et al.*, 1995)	200	16
Seine River basin, France (Motelay-Massei *et al.*, 2004)	450- 5650	14	Ulsan (industrial city, Korea) (Doong & Lin, 2004)	11840- 245120	16
Taragona County, Spain (Nadal *et al.*, 2004)	166-1002	16	Shanghai Industrial Area (Liu *et al.*, 2007)	14247 (summer) 31163 (winter)	16
This study	2804 ± 2645 - 18384 ± 12324	15	This study	3056 ± 1929 - 13581 ± 5295	15
Commercial Area					
Miami, Florida (Banger *et al.*, 2010)	*2364*	16	City Centre of Newcastle upon Tyne, north east England (Lorenzi *et al.*, 2011)	590- 46000	16
Urban USA (Mauro *et al.*, 2006)	84- 147000	43	Guangzhou, China (Wang *et al.*, 2011)	840- 12300	16
This study	25979 ± 39357– 333039 ± 623112		Ulsan City (Dong and Lee, 2009)	45750 ± 24660	16
		15	This study	32837 ± 29186 – 329299 ± 166569	15
Residential Area					
Beijing, China (Tang *et al.*, 2005)	365- 5284	16	Egypt (Mostafa *et al.*, 2009)	27.0- 76.0	30
Miami, Florida (*residential and public parks* areas) (Banger *et al.*, 2010)	1508–1595	16	This study	41984 ± 39623– 1043245 ± 156396	15
This study	1580 ± 2010- 49699 ± 45760	15			

Table 3.14 Comparison between PAHs in road dusts and road side soils of various areas in Malaysia

Soil			Road Dust		
Location	**No. of PAHs**	**Concentration (µg kg^{-1} dw)**	**Location**	**No. of PAHs**	**Concentration (µg kg^{-1} dw)**
Urban Kuala Lumpur (Omar *et al.*, 2002)	17	224± 108	Kuala Lumpur (Zakaria *et al.*, 2002)	15	1080- 4550
Urban Kemamam (Tahir *et al.*, 2008)	16	6.30 - 176			
Urban Kota Bharu (Fadzil *et al.*,2008)	16	22 - 24060			

3.3.4 PAH Pattern in road side soils

3.3.4.1 Industrial areas

The composition of PAHs found in road side soils of industrial areas can be represented based on ring number (Fig. 3.4). The PAHs in the Chan Saw Lin area are dominated by 4 ring compounds at 35 %, followed by 6 (26 %), 5 (22 %), and 3 ring (16 %). On the other hand, in Kuchai soils 3 ring compounds were dominant (34 %) followed by 4 (30 %), 6 (20 %), and 5 ring PAHs (17 %). The other sampling locales had intermediate PAH distributions as can be discerned from the plot (Fig. 3.4a). In general, the PAH distributions based on ring number are diverse among locations showing no pattern.

Moreover, the relationship between high molecular weight (HMW) and low molecular weight (LMW) PAHs in the soils of all sampling locations have the HMW PAHs dominant (Fig. 3.4b). The highest level is in Chan Saw Lin (84 %) and the lowest in Kuchai (66 %). These results suggest that all the industrial areas have been contaminated by deposition of PAHs from vehicular emissions (Agarwal, 2009), and their sources are from the surrounding areas because an elevated HMW distribution indicates regional emissions (Yang *et al.* 1991; Meharg *et al.* 1998).

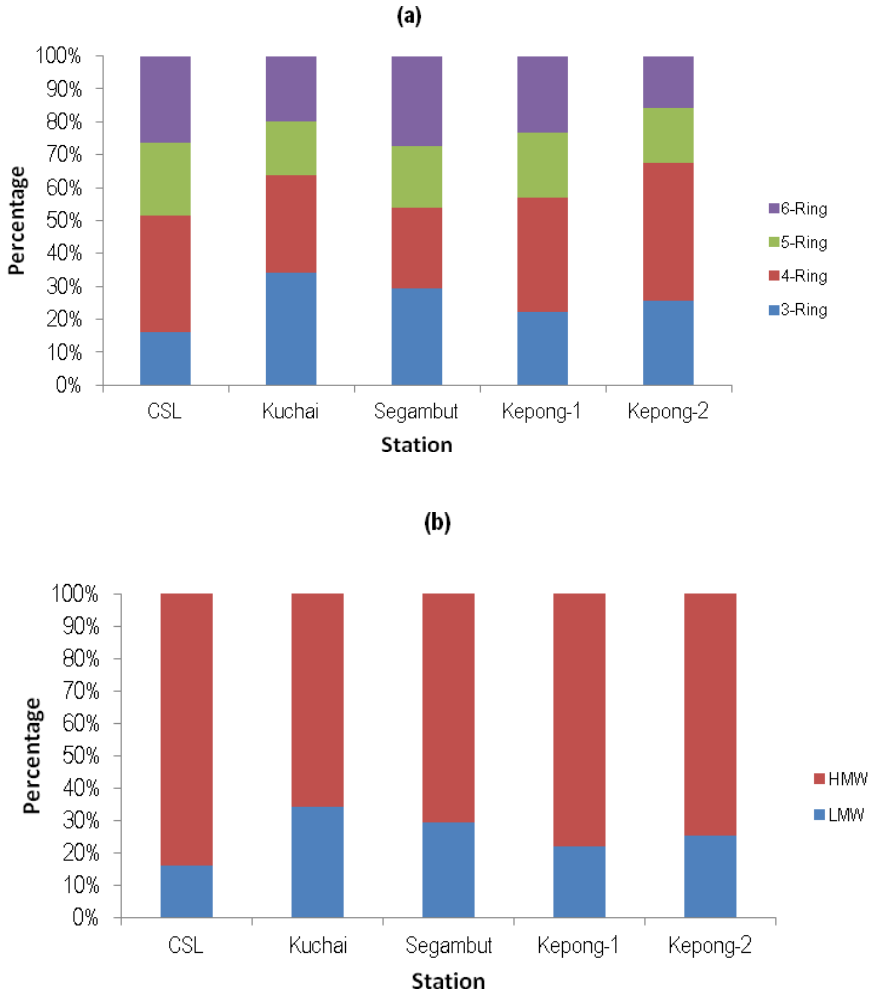


Figure 3.4 (a) Plot of PAH distributions based on ring number in road side soils of the industrial areas; (b) Plot of high molecular weight (HMW) vs. low molecular weight (LMW) PAHs in road side soils of the industrial areas

3.3.4.2 Commercial areas

As for the commercial areas, Fig. 3.5a shows that PAHs in Ampang Park is dominated by 6 ring compounds with 48 % followed by 5 ring (31 %), 4 ring (19 %) and 3 ring (2.1 %). As for Sultan Ismail: 6 ring (61 %) > 5 ring (20 %) > 4 ring (17 %) > 3 ring (2 %). Meanwhile, for Masjid India: 6 ring (57 %) > 5 ring (23 %) > 4 ring (18 %) > 3 ring (2 %). In Bukit Bintang: 6 ring (57 %) > 5 ring (21 %) > 4 ring (18 %) > 3 ring (3 %). Imbi recorded 6 ring (63 %) > 5 ring (18 %) > 4 ring (16 %) > 3 ring (3 %) while Raja Chulan recorded 6 ring (57 %) > 4 ring (21 %) > 5 ring (19 %) > 3 ring (3 %). Overall, the result shows that all

sampling sites for the commercial areas are dominated by 6 ring PAHs which supports the identification of indeno[1,2,3- cd]pyrene as the most abundant PAH compounds found in the commercial areas.

As for the ratio between high molecular weight (HMW) PAHs to low molecular weight (LMW) PAHs in road side soils of commercial areas (Fig. 3.5b), it is observed that HMW PAHs dominate in all sampling sites with 90 % in Ampang Park, 88 % in Sultan Ismail, 89 % in Masjid India, 84 % in Bukit Bintang, 83 % in Imbi and 88 % in Raja Chulan.

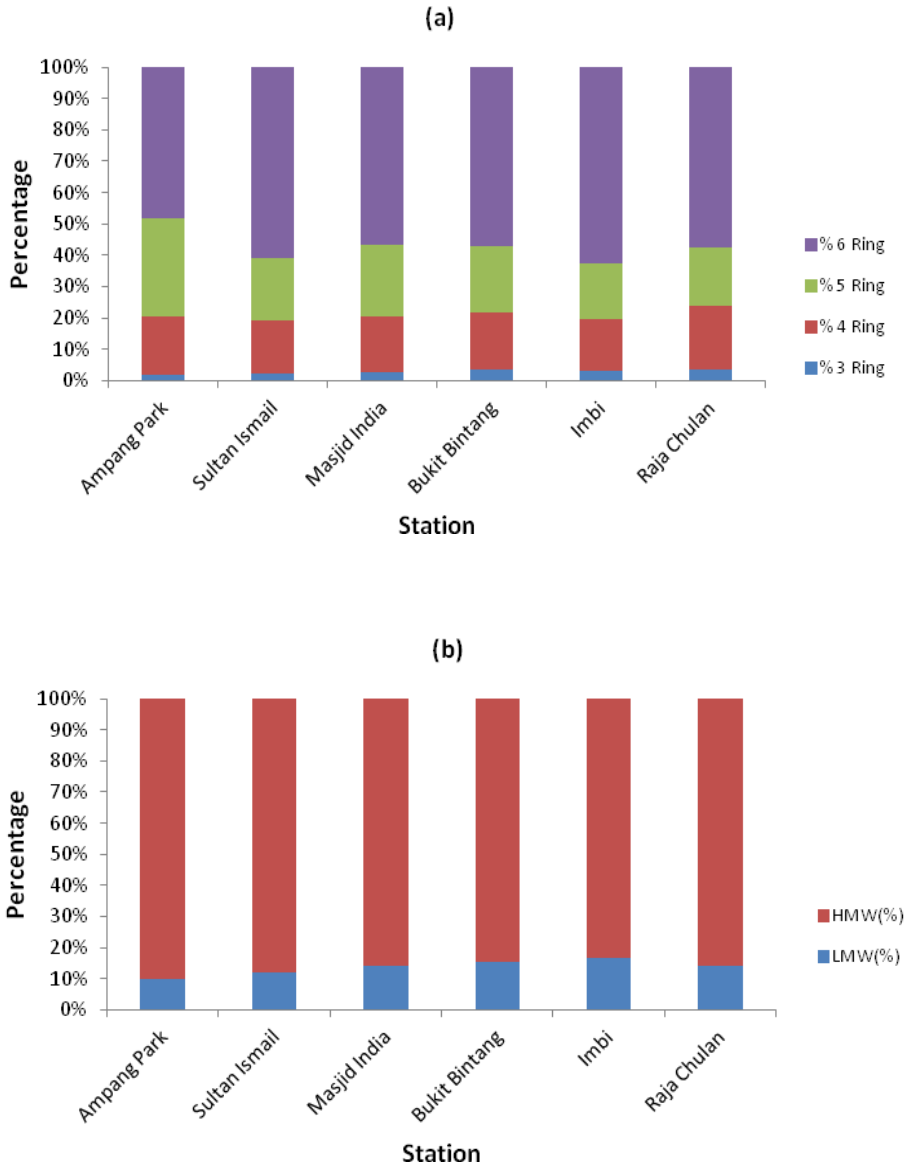


Figure 3.5 (a) Plot of PAH distributions based on ring number in road side soils of the commercial areas; (b) Plot of high molecular weight (HMW) vs. low molecular weight (LMW) PAHs in road side soils of the commercial areas

3.3.4.3 Residential areas

For the residential areas (Fig. 3.6a), the trends at all the sampling locations are about the same as in the commercial areas. As for U- Thant: 6 ring (56 %) > 5 ring (20 %) > 4 ring (19 %) > 3 ring (5 %). For Kg. baru: 6 ring (63 %) > 5 ring (19 %) > 4 ring (14 %) > 3 ring (4.10 %). For Segambut: 6 ring (56 %) > 5 ring (23%) > 4 ring (18 %) > 3 ring (4 %). For PPR Sg. Besi: 6 ring (48 %) > 4 ring (31 %) > 5 ring (18 %) > 3 ring (4 %). As for Puncak

Jalil: 6 ring (41 %) > 4 ring (31 %) > 5 ring (14 %) > 3 ring (14 %). Meanwhile for Jln. Datuk Sulaiman, TTDI: 6 ring (57 %) > 4 ring (20 %) > 5 ring (15 %) > 3 ring (8 %). The trend of PAHs domination in U- Thant and Kg. Baru is the same as their nearest commercial area (Ampang Park) while for Segambut residential area and PPR Sg. Besi, the trend is different from their nearest industrial areas (Segambut industrial area and Chan Saw Lin industrial area). The difference suggests that the studied residential area which is located next to industrial area is not affected by the activities in the industrial areas.

Overall, HMW PAHs are still the dominant PAHs in all the studied residential areas. In U- Thant, HMW PAHs recorded was 80 % of the total PAHs recorded while Kg. Baru was 78 %, Segambut with 82 %, PPR Sg. Besi with 89 %, Puncak Jalil with 69 % and Jln. Datuk Sulaiman with 71 %.

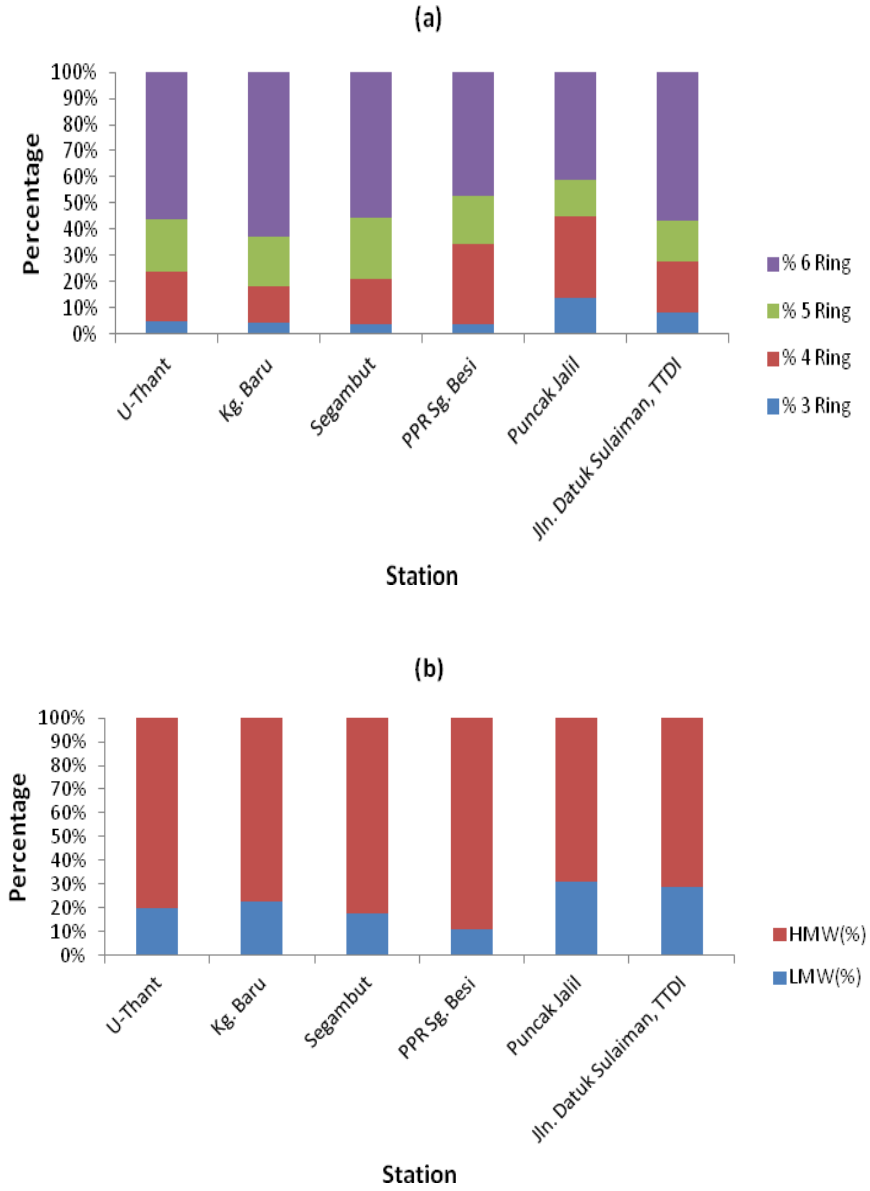


Figure 3.6 (a) Plot of PAH distributions based on ring number in road side soils of the residential areas; (b) Plot of high molecular weight (HMW) vs. low molecular weight (LMW) PAHs in road side soils of the residential areas

3.3.5 PAHs pattern in road dusts

3.3.5.1 Industrial areas

The PAH percentage based on ring number in the road dusts is shown in Fig. 3.7a. The results were similar, e.g. Chan Saw Lin had 4 rings (37 %) > 6 (23 %) > 3 (21 %) > 5 (20

%). Unlike the PAH distributions in the road side soils, these show the major contribution by 4 ring PAHs, except for Segambut where 3 ring PAHs dominated.

The plot of HMW to LMW PAHs in the road dusts is shown in Fig. 3.7b. HMW PAHs dominate Chan Saw Lin (80 %) as maximum and Kepong- 1 (70 %) as minimum. Only Segambut is dominated by LMW PAHs at 62 %. The situation in the Segambut industrial area suggests that short- range atmospheric transport i.e. local input was the biggest source of PAHs in that area.

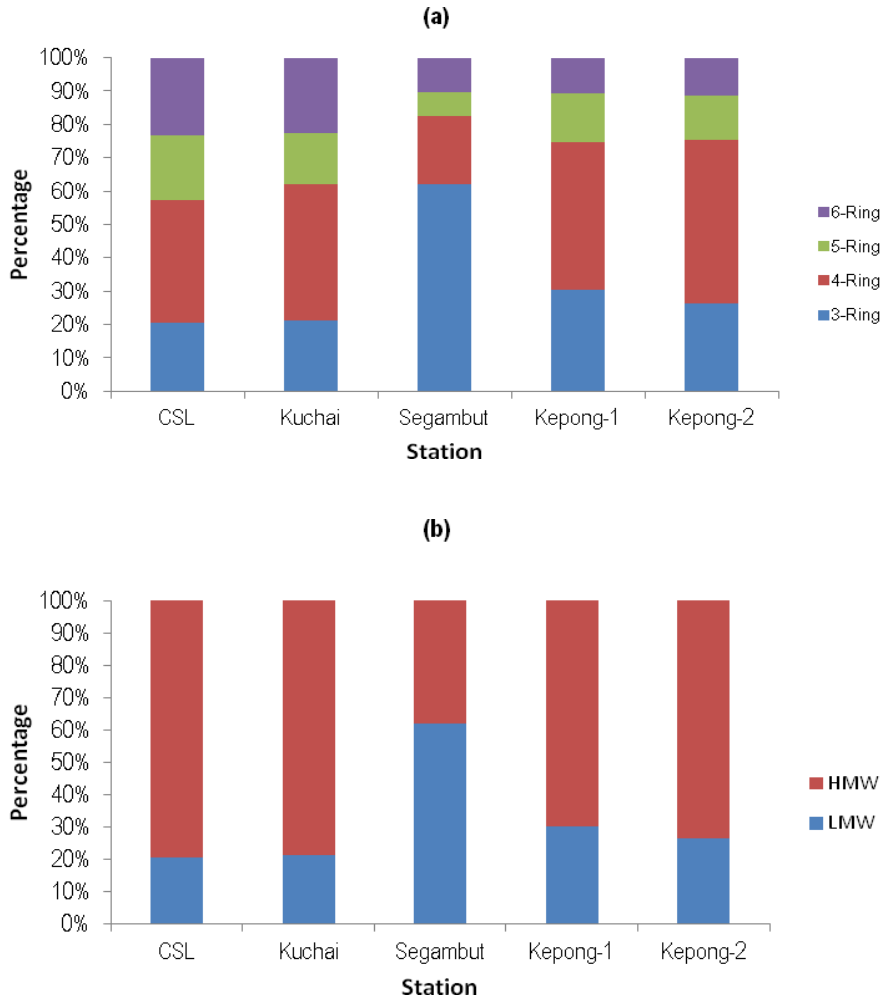


Figure 3.7 (a) Plot of PAH distributions based on ring number in road dusts of the industrial areas; (b) Plot of high molecular weight (HMW) vs. low molecular weight (LMW) PAHs in road dusts of the industrial areas

3.3.5.2 Commercial areas

The distribution of PAHs in road dusts of the studied commercial areas based on their number of rings are as follows; Ampang Park: 6 ring (67 %) > 5 ring (21 %) > 4 ring (11 %) > 3 ring (1 %), Sultan Ismail: 6 ring (59 %) > 5 ring (22 %) > 4 ring (17 %) > 3 ring (2 %), Masjid India: 6 ring (52 %) > 4 ring (23 %) > 5 ring (22 %) > 3 ring (3 %), Bukit Bintang: 5 ring (35 %) > 4 ring (31 %) > 6 ring (30 %) > 3 ring (4 %), Imbi: 6 ring (70 %) > 5 ring (16 %) > 4 ring (12 %) > 3 ring (2 %), Raja Chulan: 6 ring (63 %) > 5 ring (19 %) > 4 ring (16 %) > 3 ring (2 %) (Fig. 3.8a).

Based on the graph of HMW versus LMW of the studied commercial areas (Fig. 3.8b), it can be seen that HMW PAHs are dominant in all sampling areas with 99 % in Ampang Park, 98 % in Sultan Ismail, 97 % in Masjid India, 96 % in Bukit Bintang, 98 % in Imbi and 98 % in Raja Chulan. Extreme domination of HMW PAHs suggests that the sources of PAHs in the studied commercial areas were from high- temperature combustion such as from vehicular emissions (Mastral and Callen, 2000).

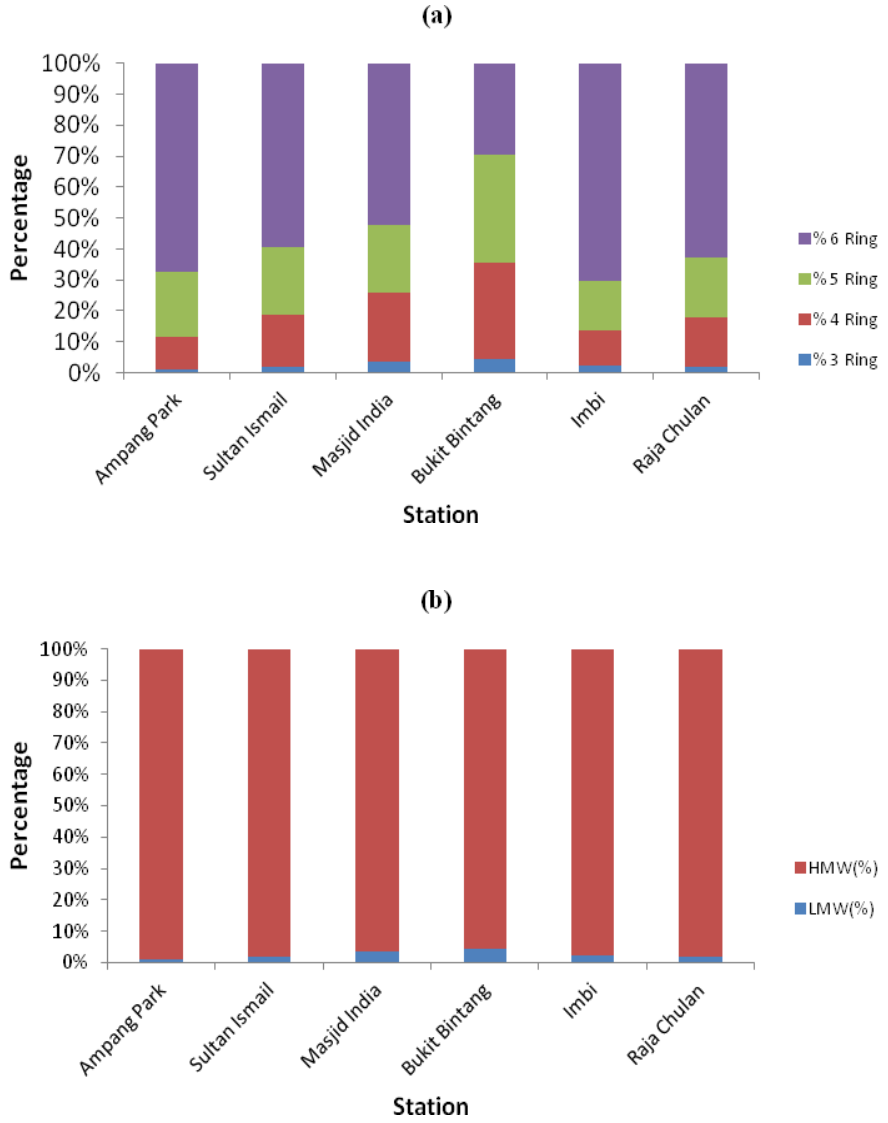


Figure 3.8 (a) Plot of PAH distributions based on ring number in road dusts of the commercial areas; (b) Plot of high molecular weight (HMW) vs. low molecular weight (LMW) PAHs in road dusts of the commercial areas

3.3.5.3 Residential areas

Distribution of idividual PAHs in residential areas are as follows; In U- Thant: 6 ring (55 %) > 5 ring (23 %) > 4 ring (20 %) > 3 ring (2 %), Kg. Baru: 6 ring (56 %) > 5 ring (24 %) > 4 ring (18 %) > 3 ring (2 %), Segambut: 6 ring (63 %) > 5 ring (19 %) > 4 ring (15 %) > 3 ring (2 %), PPR Sg. Besi: 6 ring (68 %) > 5 ring (21 %) > 4 ring (10 %) > 3 ring (1 % ring), Puncak Jalil: 6 ring (55 %) > 5 ring (30 %) > 4 ring (13 %) > 3 ring (1 %), Jln. Datuk

Ismail, TTDI: 6 ring (56 %) > 4 ring (21 %) > 5 ring (20 %) > 3 ring (3 %) (Fig. 3.9a). The trend of individual distribution of PAHs in road dusts of residential areas are the same as in road side soils of residential areas suggesting that both road side soils and road dusts originated from the same sources.

High molecular weight (HMW) PAHs are also the dominant PAHs in all studied areas (Fig 3.9b). The percentages of HMW PAHs recorded are 98 % in U- Thant, 98 % in Kg. Baru, 98 % in Segambut, 99 % in PPR Sg. Besi, 99 % in Puncak Jalil and 97 % in Jln. Datuk Sulaiman. These results suggest that pyrogenic activities as the main sources of PAHs (Mastral and Callen, 2000; Morillo *et al.*, 2007; Wilcke, 2007).

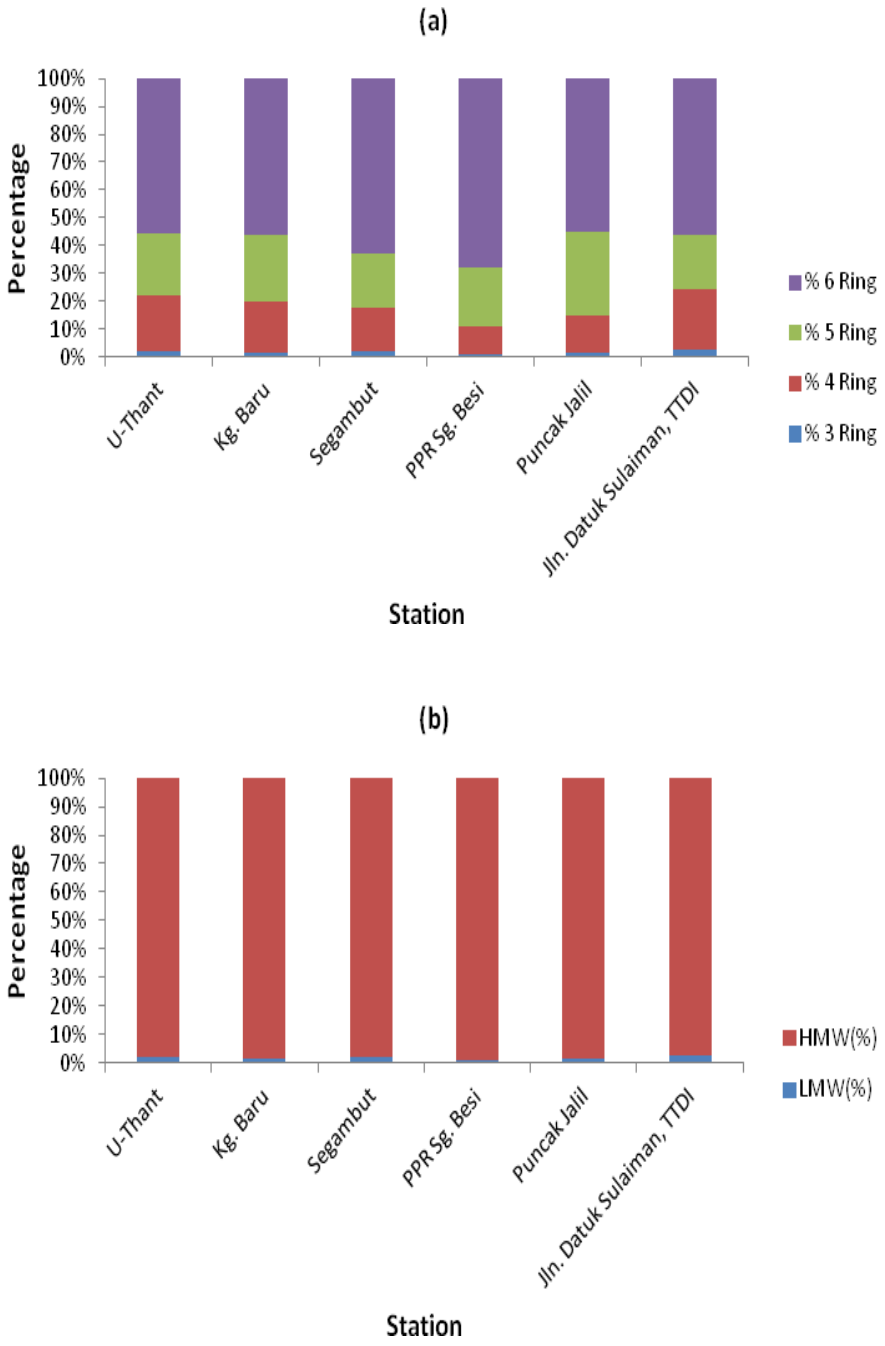


Figure 3.9 (a) Plot of PAH distributions based on ring number in road dusts of the residential areas; (b) Plot of high molecular weight (HMW) vs. low molecular weight (LMW) PAHs in road dusts of the residential areas

3.3.6 Relationship between PAHs in road side soils and road dusts

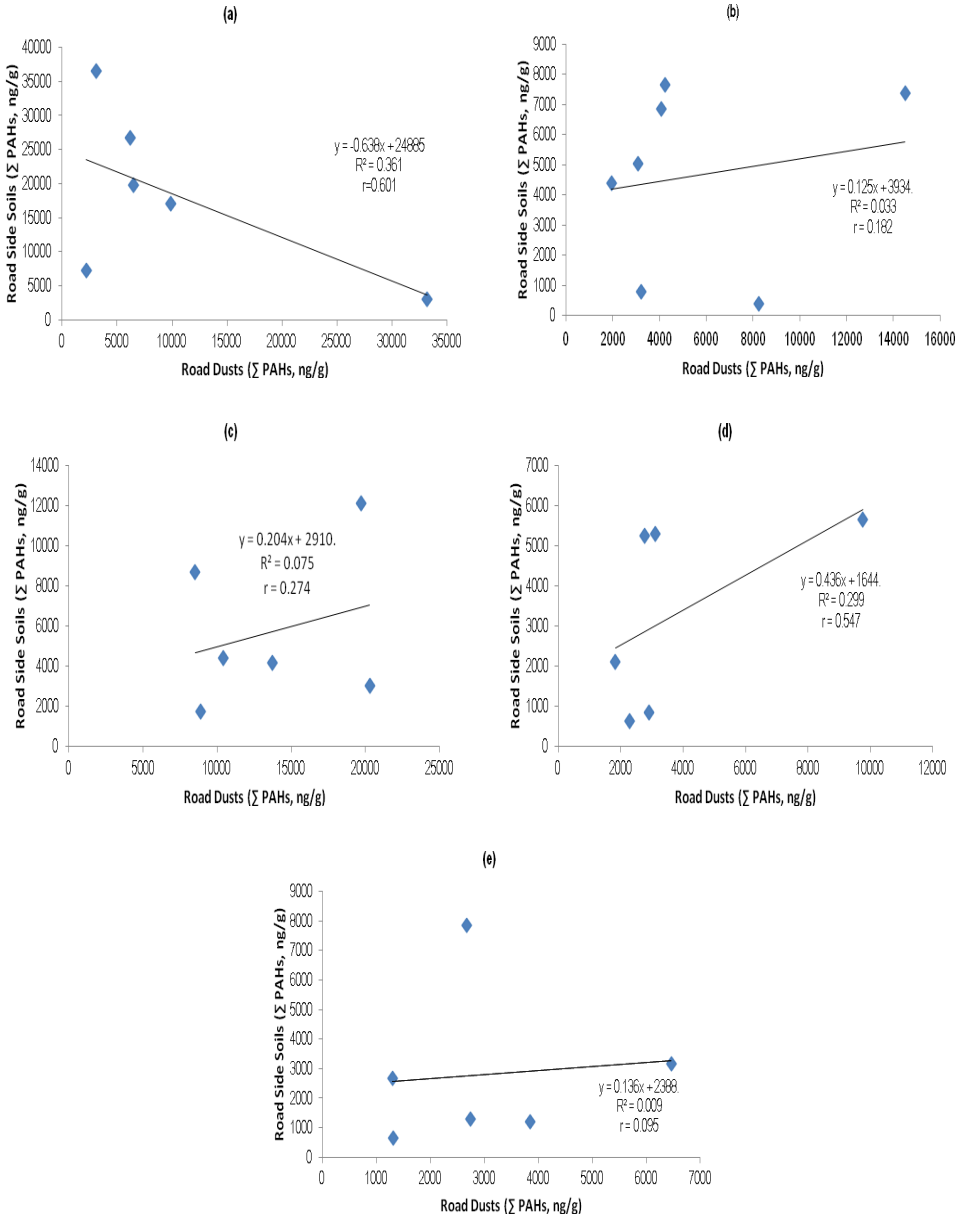


Figure 3.10 Correlation plots of PAHs between road side soils and road dusts of the industrial areas: a) Chan Saw Lin, b) Kuchai, c) Segambut, d) Kepong- 1 and e) Kepong- 2

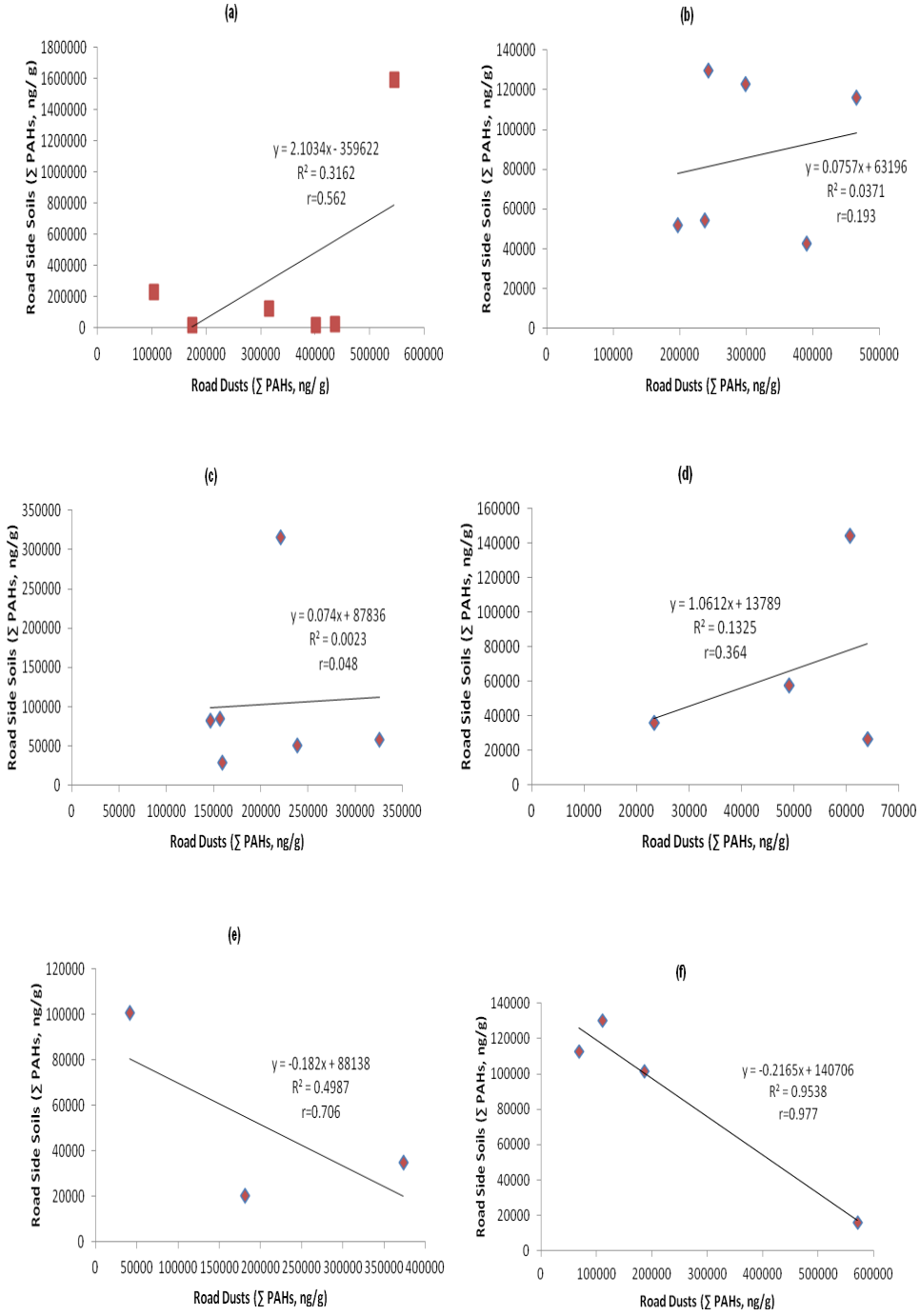


Figure 3.11 Correlation plots between road side soils and road dusts of the commercial areas: a) Ampang Park, b) Sultan Ismail, c) Masjid India, d) Bukit Bintang, e) Imbi and f) Raja Chulan

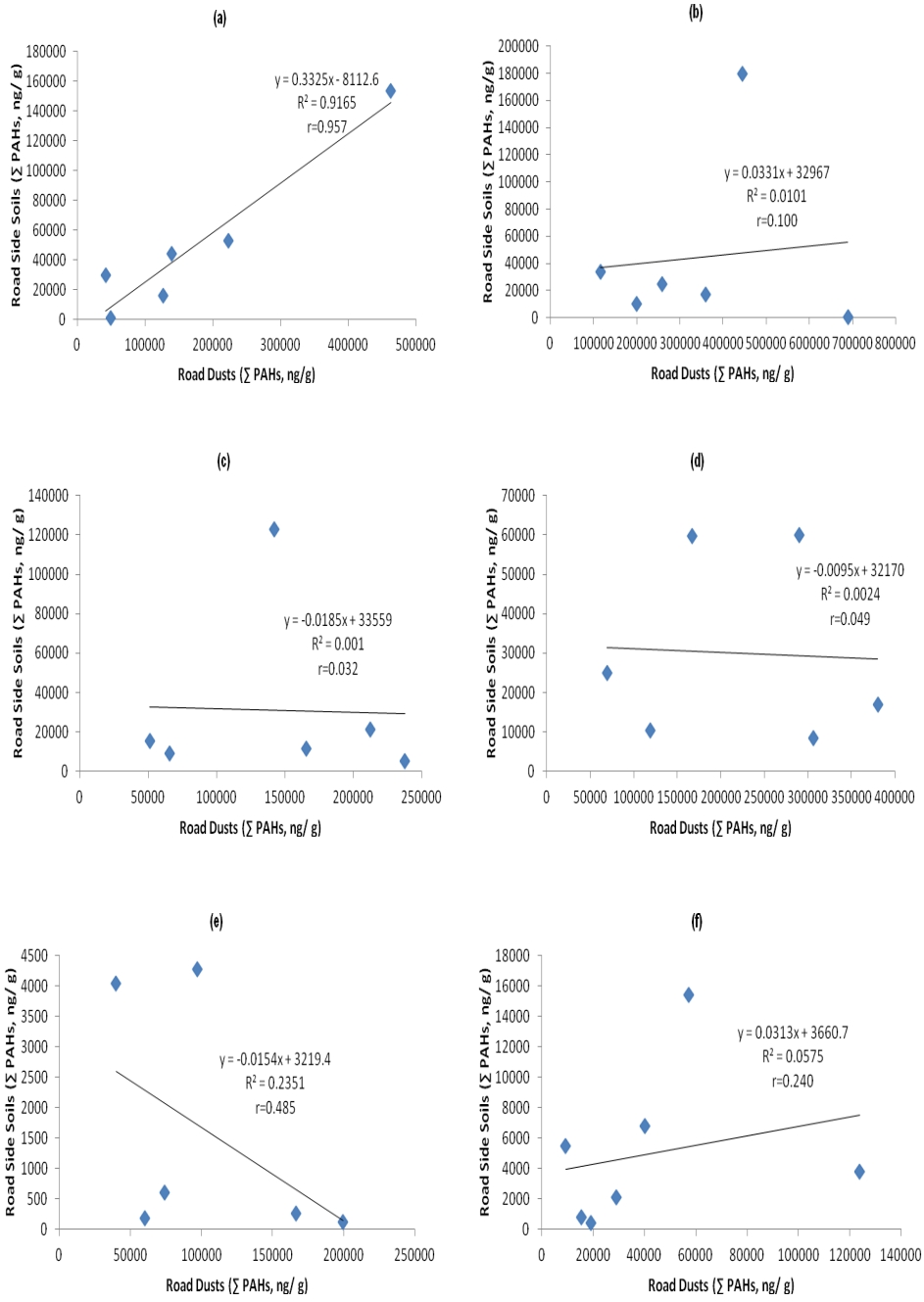


Figure 3.12 Correlation plots of PAHs between road side soils and road dusts of the residential areas: a) U- Thant, b) Kg. Baru, c) Segambut, d) PPR Sg. Besi, e) Bukit Jalil and f) Jln. Datuk Sulaiman, TTDI

The correlations of PAHs in road side soils versus road dusts were highly variable among the locations (Fig. 3.10, 3.11, and 3.12). The correlation factors varied from high for Raja Chulan (r= 0.98) and U- Thant (r= 0.96), moderate for Chan Saw Lin (r= 0.60), Kepong- 1

(r= 0.55), Ampang Park (0.56) and Bukit Jalil (0.49), to low for Segambut (r= 0.27), Bukit Bintang (r= 0.364), and Jalan Datuk Sulaiman (r= 0.24), and negligible for Kuchai (r= 0.18), Kepong- 2 (r= 0.10), Sultan Ismail (r= 0.19), Masjid India (r= 0.05), Kg. Baru (0.10), Segambut residential area (0.03) and PPR Sg. Besi (0.05). Overall, from the results obtained, it can be said that the relationship between PAHs in road side soils and road dusts varies and depending on the sampling locations itself. It does not depend on land use as the results shows that the relationship varies even if it has the same land uses characteristics.

3.3.7 Sources of PAHs

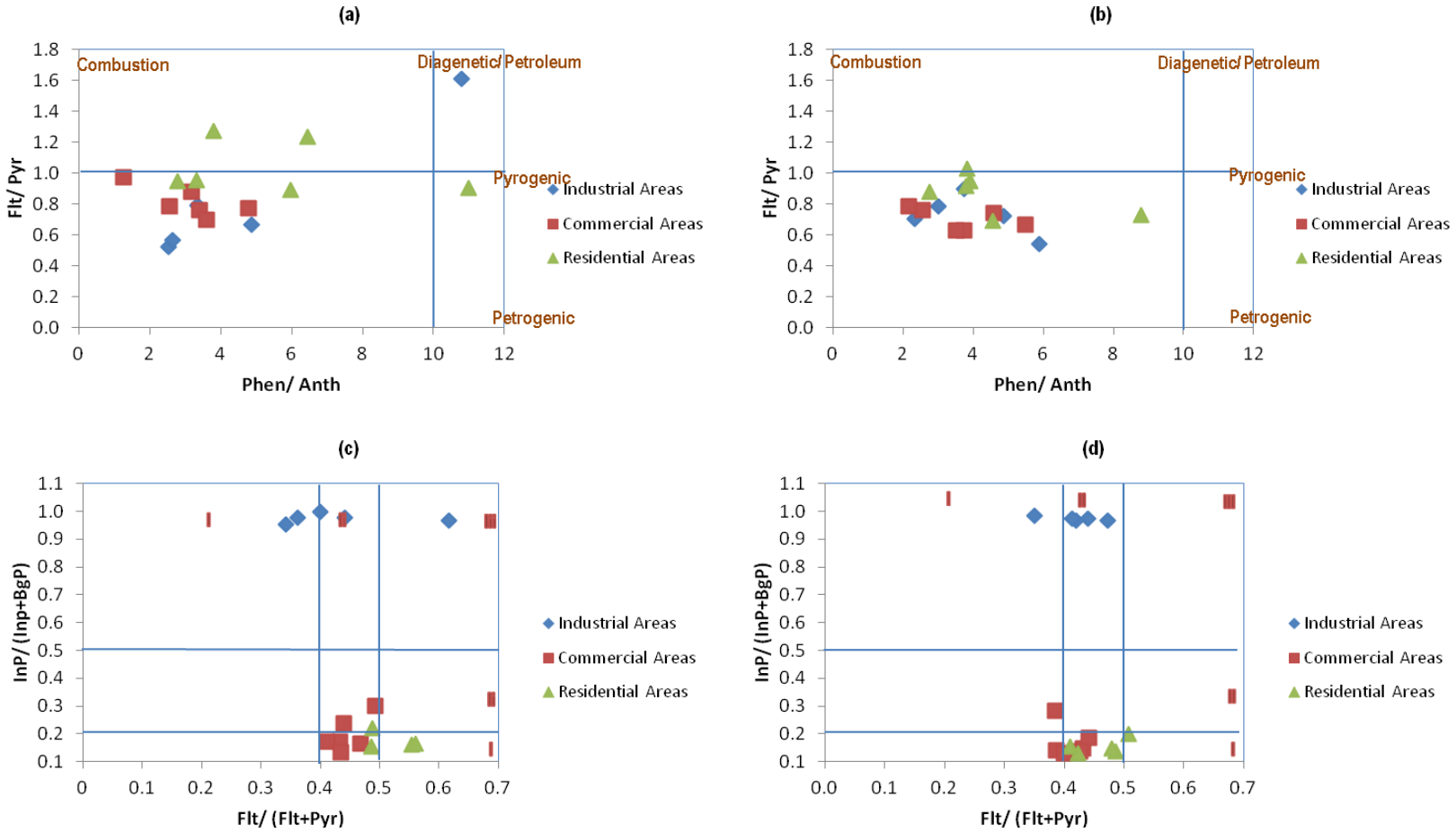
PAHs can be derived from natural and anthropogenic sources. Examples of natural sources of PAHs are forest fires, natural petroleum seepage and bituminous shales (Simoneit and Fetzer 1996). However, most of the PAH sources in urban environments are anthropogenic and can be divided into two categories, i.e. pyrogenic (combustion of biomass and fossil fuels) and petrogenic (input of petroleum products and crude oil).

The abundance of PAHs with certain ring numbers can indicate a general origin for their sources. PAHs enriched in 3 and 4 ring compounds are known to derive from petroleum residues (Tolosa *et al.*, 1996), while PAHs enriched in higher rings derive from combustion processes (Irwin *et al.*, 1997; Huang *et al.*, 2003). From the results, since the amount of 3 and 4 rings PAHs in road side soils and road dusts of industrial areas are greater or equal to 50% of the total concentrations of PAHs, it is concluded that industrial areas of Kuala Lumpur city received greater amount of petrogenic PAHs than pyrogenic PAHs. High amounts of petrogenic PAHs in all sampling stations might have come from petroleum residues from workshops found around the sampling locations. However, in commercial and residential areas, it has been shown that 5 and 6 ring PAHs are dominating, suggesting pyrogenic activities as the sources. The main activity that clearly contributes to

pyrogenic PAHs is vehicular traffic through exhaust emissions (Pereira Netto *et al.*, 2006). Exhaust emissions may however vary with traffic volume, vehicle speed and the type of the road surface (Mi *et al.*, 2001; Dong and Lee, 2009). Previous studies have shown the connection between PAH levels with heavy traffic areas (Takada *et al.*, 1991; Pereira Netto *et al.*, 2006). A study by Mi *et al.* (2001) has reported that increasing vehicle speed will help in dispersing PAHs emissions in the atmosphere.

Correlations of ratios of individual PAHs can also be used to elucidate sources of PAHs. Fig. 3.13 (a) and (b) shows the correlations between Flt/ Pyr and Phen/ Anth where a value of Flt/ Pyr < 1 suggests that these compounds derived from petrogenic sources and > 1 from pyrogenic emissions (Doong and Lin, 2004). Conversely, a value for Phen/Anth < 10 suggest that these PAHs originated from combustion processes and > 10 from diagenetic processes or from petroleum sources (Baumard *et al.*, 1998). The values of Flt/ Pyr for industrial, commercial and residential road dust samples and most of the road side soil samples were < 1 and those for Phen/ Anth were < 10 for most of the samples (Fig. 3.13a,b). PAH sources can also be elucidated by correlating the ratios of InP/ (InP + BgP) against Flt/ (Flt + Pyr) (Fig. 3.13c,d). A value for Flt/ (Flt + Pyr) < 0.4 indicates that these PAHs originated from petroleum pollutants, at 0.4 is interpreted as the transition to petroleum combustion processes, while > 0.5 supports an origin from wood or coal combustion (Banger *et al.*, 2010). On the other hand, a value for InP/ (InP + BgP) < 0.2 indicate that these compounds originate directly from petroleum, > 0.5 for wood and coal combustion, while between 0.2 and 0.5 suggests an input from petro- chemical fuel combustion (Yunker *et al.* 2002; Luo *et al.* 2005). As for the Flt/ (Flt + Pyr) ratios for road side soils samples, the values range from 0.342 to 0.617 in industrial areas, 0.411 to 0.493 in commercial areas and 0.471 to 0.560 in residential areas. Meanwhile, for the road dusts, the ratios range from 0.351 to 0.473 in industrial areas, 0.385 to 0.441 in commercial areas

and from 0.410 to 0.507 in residential areas. Referring to Fig. 3.13 (c) and (d), it can be seen that the trend of sources identification for both road side soils and road dusts were about the same. The Flt/ (Flt + Pyr) results suggest that industrial areas have been affected by all petroleum pollutants, transition of petroleum/ combustion process and by wood or coal combustions. As for the commercial areas, the result shows that all of the studied commercial areas came from transitional process of both petroleum and combustion activities. For the residential areas, the results shows that PAHs of all studied residential areas came from transition of petroleum/ combustion processes and the combustion of wood or coal. Meanwhile, for InP/ (InP + BgP), it can be seen that the ratios in road side soils range from 0.966- 1.00 for industrial areas, 0.134- 0.300 for commercial areas and 0.008- 0.220 in residential areas. For the road dusts, the ratios recorded ranged from 0.968- 0.984 in industrial areas, 0.131- 0.284 in commercial areas and 0.070- 0.199 in residential areas. Industrial areas have recorded the primary contribution of PAHs through wood and coal combustion through this ratio assessment while commercial areas being affected by petroleum discharge and petro- chemical fuel combustion. On the other hand, the ratios of InP/ (InP + BgP) show that PAHs in road side soils of the studied residential areas were affected by both petroleum discharge and petro- chemical fuel combustion as well while it road dusts were affected mainly by petroleum input or discharged.



Legend: **I**= Petroleum; **II**= Petroleum Combustion; **III**= Biomass and Coal Combustion

Figure 3.13 (a) and (b): Correlation plot for the isomeric ratios of Phen/ Anth vs. Flt/ Pyr ((a) for road side soil while (b) for road dusts), and, (c) and (d): correlation plot for the isomeric ratios Flt/ (Flt + Pyr) vs. InP/ (InP + BgP) ((c) for road side soil while (d) for road dusts)

3.3.8 Principal component analysis

Principal component analysis (PCA) was performed to further examine the PAH sources. PCA is a multivariate diagnostic tool used to shrink a set of original variables and produce a small set of dormant factors to analyze relationships among the variables. The variances for the first two principal components (PC 1 and PC 2) for these samples accounted for 98 % (Fig. 3.14a,b). The variables number of each sampling sites for this study are listed in Table 3.15.

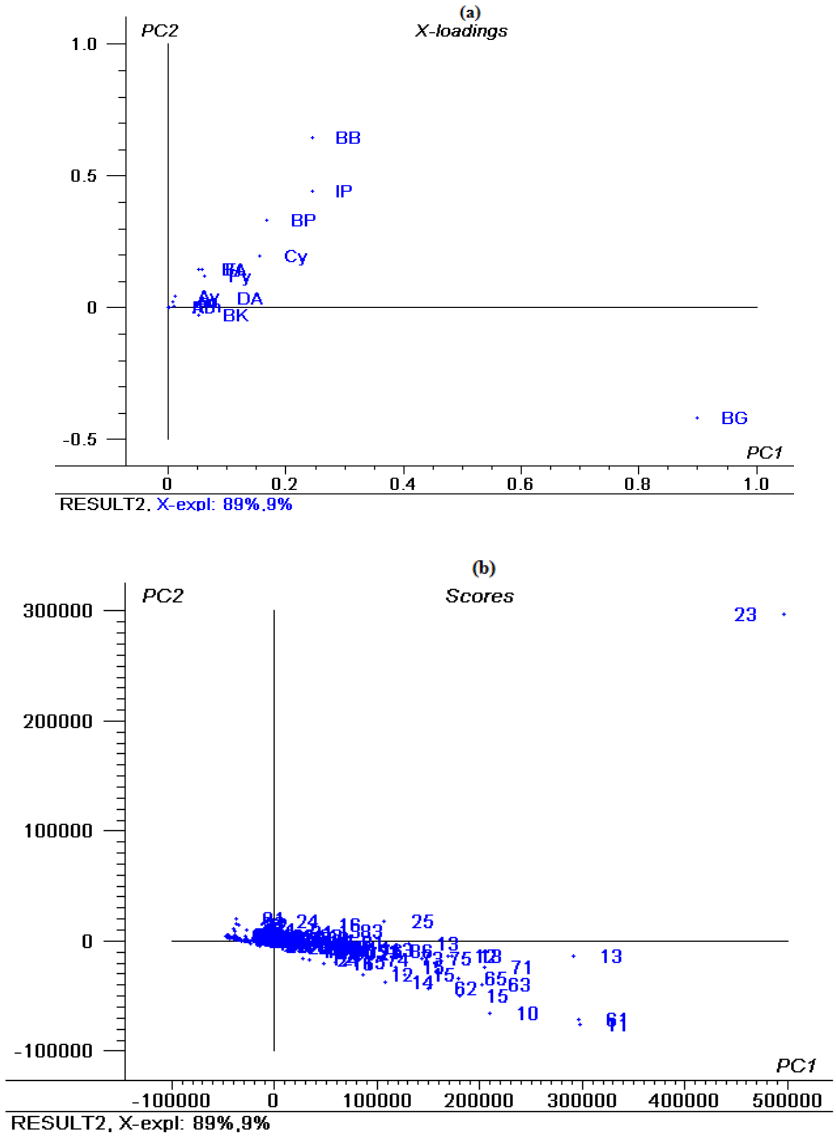


Figure 3.14 Plots with PC 1 and PC 2 from principal component analysis. (a) Factor loadings of 15 PAHs on PC 1 and PC 2, and (b) factor scores of 194 sampling sites on the PC 1 and PC 2

Table 3.15 Variables number for each sampling sites for the Principal Component Analysis (PCA) studies

Studied Areas	Road Side Soils Variables	Road Dusts Variables
Industrial Areas		
Chan Saw Lin	11- 16	181- 186
Kuchai	21- 27	191- 197
Segambut	31- 36	201- 206
Kepong 1	41- 46	211- 216
Kepong 2	51- 56	221- 226
Commercial Areas		
Ampang Park	61- 66	231- 236
Sultan Ismail	71- 76	241- 246
Masjid India	81- 86	251- 256
Bukit Bintang	91- 94	261- 264
Imbi	101- 103	271- 273
Raja Chulan	111- 115	281- 285
Residential Areas		
U-Thant	121- 126	291- 296
Kg. Baru	131- 136	301- 306
Segambut	141- 146	311- 316
PPR Sg Besi	151- 156	321- 326
Puncak Jalil	161- 166	331- 336
Jln. Datuk Sulaiman	171- 177	341- 346

PC 1 indicates 89 % of the total variance while PC 2 indicates 9 % of it. PCA results show that low molecular weight PAHs has a very low contribution to both principle components. Higher loadings of high molecular weight PAHs fluoranthene (Flt), pyrene (Pyr), benz[a]anthracene (BA), chrysene (Cy), benzo[b]fluoranthene (BB), benzo[k]fluoranthene (BK), benzo[a]pyrene (BP), dibenz[a,h]anthracene (DA), benzo[g,h,i]perylene (BG) and indeno[1,2,3- cd]pyrene (IP) were identified in this factor (Fig. 3.14a) which are common markers of pyrolysis or incomplete combustion. Benzo[a]pyrene and benzo[g,h,i]perylene are suitable tracers of vehicle exhaust emissions as they were abundant in traffic tunnels (Harrison *et al.* 1996, Larsen and Baker, 2003). Therefore, it is believed that gases releases from vehicles are the main contributor of PAHs in Kuala Lumpur city as benzo[g,h,i]perylene has been found to be the greatest contributor of PAHs in road side soils and road dusts of most of studied areas. On the other hand, fluoranthene, pyrene, benz[a]anthracene, chrysene and

benzo[a]pyrene can be used as markers for coal combustion (Simcik *et al.*, 1999; Larsen and Baker, 2003; Duval and Friedlander, 1981). Meanwhile, is also has been found that the combustion of both diesel and natural gas result in the release of benz[a]anthracene and chrysene (Rogge *et al.*, 1993; Khalili *et al.*, 1995). The presence of benzo[b]fluoranthene and benzo[k]fluoranthene support a contribution from fossil fuel combustion as these two components with the others have been found in the emission products (Rogge *et al.*, 1993; Kavouras *et al.*, 2001). The presence of indeno[1,2,3-cd]pyrene which can indicate a contribution from diesel vehicles (Rogge *et al.*, 1993; Kavouras *et al.*, 2001), while dibenz[a,h]anthracene together with indeno[1,2,3-cd]pyrene and benzo[a]pyrene indicate several traffic emissions (Fraser *et al.*, 1997). It should be noted that there are no uniquely exclusive PAH tracers for fossil fuel, natural and pyrogenic sources, only some PAHs stand out specifically among all compounds in sources. Overall, both principal components shows that pyrogenic sources were the main origin of PAHs in road dusts and road side soils with higher concentrations observed in Kuchai (Fig. 3.14b) which has high traffic densities and numerous petroleum- related activities.

PCA results have shown that LMW PAHs has very limited effects on the distribution trend. Among LMW PAHs in the environment, it has also been reported that phenanthrene (Ph), anthracene (An) and fluorene (Fl) can be produced by coke ovens (Duval and Friedlander, 1981) while fluoranthene (Ft) and pyrene (Py) is a marker for coal combustion (Larsen and Baker, 2003). These findings indicate that coke oven and coal combustion has a very low impact on the distribution of PAHs in Kuala Lumpur city especially in the road side soils and the road dusts.

3.3.9 Risk assesment

Humans may become exposed to contaminated soil or dust mainly by incidental ingestion and inhalation. PAHs especially BaP has been proven to cause tumors in

laboratory animals from inhaling the compounds, skin contact or ingesting the compounds. On the other hand, studies by ATSDR (1995) have shown that long term exposure of humans to PAHs in any mixture by inhalation and skin contact can also cause cancer. According to the USEPA (1986), taking 0.30 mg of anthracene, 0.06 mg of acenaphthene, 0.04 mg of fluoranthene, 0.04 mg of fluorene, and 0.03 mg of pyrene per kilogram (kg) of body weight daily does not cause any bad health effects (ATSDR, 1995; Florida Department of Health, 2010) which might be due to the rapid human metabolism.

Standard concentration limits for PAHs are sparse worldwide, and only a few recommendations or guidelines exist. In Malaysia there are still no limits for PAHs defined. In this study, the total concentrations of PAHs recorded fall within the range of a few available limits, such as the Mexican standards (0-6000 $\mu g\ kg^{-1}$) and Polish standards (200-10000 $\mu g\ kg^{-1}$). However, the concentrations recorded exceed the limits stated by the Dutch (20– 50 $\mu g\ kg^{-1}$) (Agarwal *et al.*, 2009).

PAHs have also been detected in agricultural products (ATSDR, 1995) and it was believed that one of the main routes for PAH accumulation in agricultural products was through nutrient uptake from the soil. The concentrations of PAHs in these areas studied are much higher than the typical concentrations deemed suitable in soils for agriculture (200 $\mu g\ kg^{-1}$; Wilcke, 2000). Thus, these areas are not suitable for growing vegetables or any other food products as ingestion of PAHs contaminated products may cause further PAH accumulation in humans.

3.3.9.1 Road side soil and road dust toxicities

TEFs (toxic equivalency factors) are often used to measure toxicity of environmental samples. The TEF test was also used in this study. TEFs were calculated to measure the carcinogenicity of PAHs relative to benzo[a]pyrene. According to Peters *et al.* (1999), toxicological data for only benzo[a]pyrene is sufficient to measure the carcinogenic

potential of PAHs under study. According to Duke and Albert (2007), TEF values are given in parentheses for acenaphthylene (0.001), acenaphthene (0.001), fluorine (0.001), phenanthrene (0.001), anthracene (0.01), fluoranthene (0.001), pyrene (0.001), benz[a]anthracene (0.1), chrysene (0.01), benzo[b]fluoranthene (0.1), benzo[k]fluoranthene (0.1), benzo[a]pyrene (1.0), dibenz[a,h]anthracene (1.0), benzo[g,h,i]perylene (0.01) and indeno[1,2,3- cd]pyrene (0.1). The benzo[a]pyrene-equivalent concentration (B[*a*]P_{eq}) was calculated as (Agarwal *et al.*, 2009):

Total B[*a*]P_{eq} = $\sum_i$ C_i x TEF_i Equation 3.1

Where: C_i = individual PAH concentration

TEF_i = corresponding toxic equivalency factor

The resulting total B[*a*]P_{eq} for the road side soils of industrial areas studied are as follows: Chan Saw Lin (1397 µg kg^{-1}) > Segambut (337 µg kg^{-1}) > Kuchai (239 µg kg^{-1}) > Kepong- 1(198 µg kg^{-1}) > Kepong- 2 (138 µg kg^{-1}) while for commercial areas, the results are as follows: Ampang Park (52174 µg kg^{-1}) > Masjid India (17177 µg kg^{-1}) > Sultan Ismail (15241 µg kg^{-1}) > Raja Chulan (11077 µg kg^{-1}) > Imbi (9030 µg kg^{-1}) > Bukit Bintang (6266 µg kg^{-1}). As for road side soils of residential areas studied, the findings are as follows: U- Thant (4363 µg kg^{-1}) > Kg. Baru (3842 µg kg^{-1}) > Segambut (3323 µg kg^{-1}) > PPR Sg. Besi (2551 µg kg^{-1}) > Jalan Datuk Sulaiman, TTDI (470 µg kg^{-1}) > Bukit Jalil (62.7 µg kg^{-1}).

On the other hand, for the road dusts, the results are as follows; industrial areas: Chan Saw Lin (859 µg kg^{-1}) > Segambut (327 µg kg^{-1}) > Kuchai (278 µg kg^{-1}) > Kepong- 1(156 µg kg^{-1}) > Kepong- 2 (143 µg kg^{-1}), commercial areas: Sultan Ismail (48790 µg kg^{-1}) > Ampang Park (48701 µg kg^{-1}) > Masjid India (35456 µg kg^{-1}) > Raja

Chulan (27706 µg kg^{-1}) > Imbi (24856 µg kg^{-1}) > Bukit Bintang (12345 µg kg^{-1}), and residential areas: Kg. baru (40290 µg kg^{-1}) > PPR Sg. Besi (25366 µg kg^{-1}) > U- Thant (18920 µg kg^{-1}) > Bukit Jalil (14911 µg kg^{-1}) > Segambut (12288 µg kg^{-1}) > Jalan Datuk Sulaiman, TTDI (4135 µg kg^{-1}).

The trends observed for both sets were the same, where the highest carcinogenic potential is found in Chan Saw Lin and the lowest in Kepong- 2 for industrial areas. As for commercial and residential areas, the trends are different between road side soils and road dusts. Road side soils and road dusts of U- Thant and Kg. Baru (residential areas adjacent to commercial area) are found to have lower total B[*a*]P_{eq} values than their nearest commercial area (Ampang park). This trend contradics that showed in industrial areas (Chan Saw Lin and Segambut industrial area) and the residential areas adjacent to them (PPR Sg. Besi and Segambut residential area). This situation might happen due to the abundance of tall buildings in commercial areas compared to industrial areas that cause a limitation in air dispersion which traps the PAHs and causing the accumulation of it (Dong and Lee, 2009).

Total B[*a*]P_{eq} in dry soil are important to be maintained less than 370 µg kg^{-1} to protect the groundwater which serves as the raw water for drinking (Bulder *et al.*, 2006). Tsai *et al.* (2001) in their health risk study of palletizing and packaging workers in carbon black manufacturing industry towards PAHs exposure suggest that the lifetime lung cancer risks for the workers based on the B[*a*]P_{eq} were 4.34 x 10^{-2} with dermal exposure risks were 1.13 x $10^{-3}$and 1.56 x 10^{-3}, respectively. On the other hand, Vyskocil *et al.* (2004) in his study on lung cancer assessment in six localities with aluminum smelting activities exposed to PAHs, suggested that the upper bound B[a]P_{eq}-based lifetime cancer risk ranged from 0.94 to 4.7 x 10^{-5}.

3.3.9.2 Cancer risk assessment

Cancer risks to workers in the industrial areas were determined by using exposure models and risk factors developed by ATSDR (1990) and the United States Environmental Protection Agency (USEPA, 1986). Since only data on increased risk for PAHs in soil (through ingestion) are available, the determination of cancer risk for exposure to PAHs in road dusts were also determined by using related data on soil.

The formula of the oral ingestion cancer risk assessment is as follows:

Cancer risk (unitless) = Exposure dose (mg/ kg/ d) x

ingestion cancer slope factor (mg/ kg/ d)$^{-1}$) x

(estimated years exposed / BW) Equation 3.2

Assumptions:

- The contaminant concentration is the sum of the PAH TEF products and does not change from day to day. This is the maximum PAH concentration.
- Ingestion rate is 100 mg of soil per day.
- EF = Exposure Factor (unitless), exposure is assumed to be 2 d/ wk every week of the year.
- 10^{-6} kg mg^{-1} is a necessary conversion factor for soil.
- The adult body weight is 70 kg.
- Estimated years exposed = 10 years

Where:

Exposure dose (D) : (C x IR x EF x 10^{-6} kg mg^{-1}) / BW

C : contaminant concentration (mg kg^{-1})

IR : intake rate of contaminated soil (mg day^{-1})

EF : exposure factor = (2 day/ week) x (52 week/ year) x (365 day/

year) = 0.28 (unitless)

BW : body weight (kg)

EPA Ingestion Cancer Slope Factor (Florida Department of Health, 2010):

$7.30\ mg/kg/day^{-1}$

Table 3.16 Cancer risks assessment for exposure towards road side soils and road dusts of studied areas

	Cancer Risks	
Study Areas	**Road Side Soils**	**Road Dusts**
INDUSTRIAL AREAS		
CSL	6×10^{-7}	4×10^{-7}
Kuchai	1×10^{-7}	1×10^{-7}
Segambut	1×10^{-7}	1×10^{-7}
Kepong- 1	8×10^{-8}	7×10^{-8}
Kepong- 2	6×10^{-8}	6×10^{-8}
COMMERCIAL AREAS		
Ampang Park	2×10^{-5}	2×10^{-5}
Sultan Ismail	6×10^{-6}	2×10^{-5}
Masjid India	7×10^{-6}	1×10^{-5}
Bukit Bintang	3×10^{-6}	5×10^{-6}
Imbi	4×10^{-6}	1×10^{-5}
Raja Chulan	5×10^{-6}	1×10^{-5}
RESIDENTIAL AREAS		
U-Thant	2×10^{-6}	8×10^{-6}
Kg. Baru	2×10^{-6}	2×10^{-5}
Segambut	1×10^{-6}	5×10^{-6}
PPR Sg. Besi	1×10^{-6}	1×10^{-5}
Bukit Jalil	3×10^{-8}	6×10^{-6}
Jln. Datuk Sulaiman, TTDI	2×10^{-7}	2×10^{-6}

The results show that cancer risk for the people exposed to the road side soils of industrial areas of Kuala Lumpur ranged from 6×10^{-8} to 6×10^{-7} or 6 to 60 in 1×10^{9} people. As for exposure to the road dusts of industrial areas, the risk ranged from 6 to 40 in 1×10^{9} people. For the exposure to the road side soils of commercial areas, the cancer risk ranged from 3 to 20 in 1×10^{7} people while exposure to the road dusts of commercial areas ranged from 5 to 20 in 1×10^{7} people. On the other hand, the exposure to the road side soils of the residential areas ranged in between 3 to 200 in 1×10^{9} people when the exposure to the road dusts of residential areas ranged from 2 to 20

in 1 x 10^7 people (Table 3.16). These results indicate that cancer risk in these industrial, commercial and residential areas are extremely low.

3.4 Conclusion

The concentrations of PAHs in these industrial, commercial and residential areas are high in comparison to guidelines established worldwide and as noted in the literature. Activities in industrial areas clearly show that they didn't contribute much to PAHs pollution in Kuala Lumpur city in comparison to activities in commercial and residential areas. This is in contrast with the first expectation for the industrial areas to have greatest amount of PAHs in Kuala Lumpur city. Any agricultural food production is not recommended in these areas. Study on the sources of the PAHs tells that the biggest contributor of PAHs in Kuala Lumpur city came from both petrogenic and pyrogenic sources. The toxicity risk test indicated that the PAH concentrations of these areas are low, even under chronic long term exposure, the results of this study provide baseline data on PAHs and prompt the local environmental authority to take note for improving environmental quality.

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Chapter 4

Chapter 4

Degradation of PAHs by Bacteria Consortium Isolated from Fungal Comb of Soil Termite and Contaminated Road Side Soils

4.1 Introduction

The presence of PAHs in the environment is increasingly becoming an environmental concern owing to their potential toxicity, mutagenic, and carcinogenicity characteristics (Keith & Telliard, 1979; Sato & Aoki, 2002). Although PAHs in the environment are subjected to processes such as chemical oxidation, photolysis, bioaccumulation, volatilization and adsorption, microbial degradation and transformation have been identified as the principal processes for their removal (Yuan *et al.*, 2000; Cerniglia, 1993).

Recent studies have focused on bioremediation, which involves microbial degradation and transformation of organic pollutants, due to their promising capability to remedy contaminated soil at low cost (Potin *et al.*, 2004; Antizar- Ladislao *et al.*, 2004). The use of bacteria in the degradation of PAHs has been widely reported (Haritash & Kaushik, 2009) and various bacteria that have the capability to degrade individual PAHs have been isolated and identified (e.g. Cerniglia, 1993; Safekordi and Yaghmaei, 2001). However, to date, the bacteria with the capability to degrade a broad range of PAHs have not been reported (Shafiee *et al.*, 2006).

Fritsche & Hofrichter (2008) mentioned in their study that although it has been proven that a single microbe can degrade organic pollutants, microbes work best (in a degrading pollutant) when they are in a consortium. In addition, according to the study, a complex mixture of pollutants or organic compounds requires microbial communities to work together in order to degrade the pollutant efficiently since the combination of

genetic information among the group of organisms give the best potential for degradation.

Studies on biodegradation in Malaysia, especially on the degradation of PAHs, are very limited. The bacteria with PAH- degrading capabilities that have been identified in Malaysia include phenanthrene- utilizing bacterium (AR- 3) isolated from Port Dickson's sediments (Law and Teo, 1997*), Clavibacter michiganesis ss insidiosus, Rhodococcus rhodochrus* and *Brevibacterium otitidis* isolated from petroleum sludge (Dzulkapli *et al.*, 2009) as well as *Micrococcus diversus, Rhodococus rhodochrous, Corynebacterium agropyri, Corynebacterium uroalyticum* and *Pediococcus pentosaceus* isolated from municipal sludge (Othman *et al.*, 2010).

In this study, the capability of a bacteria consortium, isolated from contaminated road side soil samples and the fungal comb of soil termites, to degrade PAHs were evaluated. Although many PAH- degraders from contaminated soils have been reported, the new combination of microbes introduced in this study is believed to response differently towards PAHs. According to Haritash and Kaushik (2009), the rate and extent of the degradation depend on the type of bacteria and various environmental factors including temperature, pH, and the presence of nutrient sources.

Soils termites are also known as "soil engineers" and are one of the main macroinvertebrate decomposers in arid and semi-arid environments. The termites exert additional impacts to the soil by constructing biostructures that alter the physical and chemical properties of the soil. Not only termites themselves are known for their ability in bioremediation, bacteria that live in the termite and its surrounding were also found to have the capability to degrade pollutants. For example, a study by Ngugi *et al.* (2005) found a bacterium, *Macrotermes michaelseni*, that was isolated from the intestinal tract of a fungi- cultivating termite and has the capability to degrade resorcinol, a phenolic compound produced naturally or through human activities (Hans, 1994). *Macrotermes*

michaelseni has also been found to have the capability to degrade both phenol and benzoic acid.

Fungus- growing termites such as *Macrotermes gilvus* can be found widely in southern Asia (Roonwal, 1970) and is among the most common termite species that can be found in the soil in Kuala Lumpur. A study by Brune (1998) has shown that this species has an enlarged hindgut that works as anaerobic digesters in which a symbiotic gut microflora depolymerizes cellulose and hemicelluloses and ferments the resulting carbohydrates to short- chain fatty acids. The species lives in a nest where young worker termites eat the plant litter and let the food pass through their gut without being digested. As a result, fecal pellets or the primary feces are produced and form a sponge-like structure known as a fungus comb. According to Ohkuma (2001), a symbiotic fungus from the genus Termitomyces grows on this structure, forming mycelia as well as white, round and asexual conidial structures called fungus nodules. Young worker termites use the fungus nodules as their food while old worker termites feed on the old senescent combs, producing the final feces. However, studies on bacteria isolated from fungus combs, especially those that focus on their degradation capability, are still scarce. Moreover, studies on the ability of bacteria that are isolated from the fungus comb of *Macrotermes gilvus* and are able to degrade PAHs have not been conducted.

The aim of this study is to identify the capability of a bacteria consortium isolated from road side soils and soil termite comb from Kuala Lumpur, Malaysia to degrade PAHs as well as to identify individual species of the bacteria involved. Data obtained from this study are crucially important in identifying new bacteria consortium that is capable to degrade PAHs.

4.2 Experimental

4.2.1 Road side soils and termite fungal comb sampling

Road side soil samples (0- 5 cm in depth) were collected from sampling station no. 4 (as per Chapter 3), in the Chan Saw Lin Industrial area. The samples were taken using a stainless steel soil auger, pooled and homogenized. Before keeping it in a glass bottle, grasses, twigs and stones (if any) were removed from the samples. The bottle was then covered with an aluminium foil with small holes gnawed on it to allow air circulation. Fresh soil samples were used for the next step after reaching the lab. Fig. 4.1 below shows location for the road side soil sampling.

Figure 4.1 Sampling area of the contaminated road side soil

The soil termite fungal comb used in this study was obtained from the termite nest near the Chemistry Department building, University of Malaya (Fig. 4.2a,b). The termite fungal comb used was also placed in a pre- cleaned glass jar capped with aluminium foil that has a few small holes and used freshly in the lab. The balance from

both samples was kept in a freezer for future used. Fig. 4.2 shows the nest and the fungal comb used in this study.

(a)

(b)

Figure 4.2 a) The nest of *Macrotermes gilvus*, b) Termite fungal comb

4.2.2 Reagent, glassware and apparatus

Acetone and dichloromethane (DCM) used were of HPLC grades, purchased from Merck (Oslo, Norway). The cleaning of all glassware used in the analytical work was carried out in six steps namely: (a) soaked in 20 % Extran MA 03 Phosphate- free

overnight, (b) washed with tap water, (c) rinsed with distilled water, d) baked in an oven at 200 °C overnight, (e) wrapped with aluminium foil, and (f) autoclaved for 15 minutes at 121 °C before use.

Apparatus or equipments used in this experiment were ES- 315 Portable Autoclave (New York, USA), an incubator from Memmert (West Germany), an orbital shaker incubator Model 718 from HOTECH (Taipei, China), a UV- 1601 spectrophotometer from Shimadzu (Duisburg, Germany) and Biolog Microstation System (California, USA).

4.2.3 Chemicals and media

Fluorene, acenaphthene, fluoranthene, chrysene, pyrene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[g,h,i]perylene and indeno[1,2,3- cd]pyrene were purchased from Sigma– Aldrich Corporation (St. Louis, MO, USA) and the purity of the PAH was 97– 99 %. The stock solution of each PAH was prepared at 500 mg L^{-1} in acetone. The concentration used was either 50 mg L^{-1}, 100 mg L^{-1} or 150 mg L^{-1}.

Mineral salt mediums (MSM) used were as described by Zajic and Supplission (1972) and Abioye *et al.* (2012). Chemicals used to prepare MSM include dipotassium hydrogen phosphate (K_2HPO_4), ammonium chloride (NH_4Cl), magnesium sulfate heptahydrate ($MgSO_4.7H_2O$), potassium dihydrogen phosphate (KH_2PO_4), and sodium chloride (NaCl) which were all products of R & M Chemicals (Essex, UK), together with iron(II) sulfate heptahydrate ($FeSO_4.7H_2O$) from Bendosen Laboratory Chemicals (Bendosen, Norway), and agar from Merck (Oslo, Norway). The nutrient agar used in this study was a product of Difco (Le Pont de Claix, France).

4.2.4 Preparation of MSM and nutrient agar

Liquid medium for this study were prepared by mixing items in Table 4.1 with 1 l of distilled water.

Table 4.1 Chemicals and its amount used in preparing the MSM

Chemical	Amount (g)
K_2HPO_4	1.8
NH_4Cl	4.0
$MgSO_4.7H_2O$	0.2
KH_2PO_4	1.2
$FeSO_4.7H_2O$	0.01
NaCl	0.1

In the event that a solid medium was required, 20 g of agar was added to produce the solid medium. The pH of the mixture was maintained at 7.4 by adding natrium hydroxide, NaOH (to increase alkalinity) of concentrated hydrochloric acid, HCl (to increase acidity). The medium were then sterilized by autoclaving it for 15 minutes at 121 °C. The medium was used freshly.

On the other hand, nutrient agar was prepared by mixing 28 g of nutrient agar powder with 1 l distilled water. The mixture were then autoclaved it at 121 °C for 15 minutes.

4.2.5 Growth Test on individual PAHs

2 g of contaminated road side soil and termite fungal comb samples were separately weighed and placed in a pre- cleaned beaker containing 100 ml of saltwater (10 ‰). The saltwater was prepared by adding 10 g of sodium chloride (NaCl) into 100 ml of distilled water. Both mixtures were then shaken for 30 minutes to extract the supernatant from the samples. Example of the obtained extract was shown in Fig. 4.3.

Figure 4.3 Extracted supernatant after 30 minutes shaking process (termite fungal comb sample)

1 ml of each prepared extract was then placed in separate test tubes that were pre- filled with 9 ml of distilled water to obtain a solution with an initial dilution factor of 10^1. The solution was then serially diluted until a 10^5 dilution was obtained.

After the serial dilution, 1 ml of the 10^1, 10^3 and 10^5 dilutions was each spread on dried agar plates layered with individual PAHs in two replicates. The concentration of the PAH used in this study was 100 mg L^{-1}. The mixture was dispersed on the plates using a glass rod- spreader. The plates were then incubated in dark conditions at 35 °C for seven (7) days. The growth of the bacteria was visually measured by comparing the incubated plates with the controls, which were an uninoculated plate and a plate without PAH.

Concurrently, the growth of the bacteria used in this study was monitored using a liquid medium. 250 ml pre- cleaned conical flasks were first pre- treated with 10 ml of 500 mg L^{-1} of individual PAHs and were shaken gently to evaporate the solvent used (acetone). The flask was then filled with 90 ml of fresh MSM medium and mixed with 10 ml of the obtained supernatant aliquot. The mixture was then shaken in an incubator at 150 rpm, 35 °C and in dark conditions. The growth was monitored daily using a

spectrophotometer at optical density (O.D.) 600 nm for about one (1) week and differences in everyday absorbance readings were recorded.

In liquid culture, the medium appears more and more cloudy as the bacteria increase in number by division. A tube of bacteria will tend to reflect light so that less light is transmitted through the tube. A spectrophotometer can measure the amount of light passing through the tube, or conversely the amount of light absorbed. These measurements of turbidity or optical density (OD) are not direct measurements of bacterial numbers, but an indirect measurement of cell biomass that includes both living and dead cells.

From the monitoring of both solid and liquid medium, the growth of bacteria was only detected in a medium with fluoranthene and pyrene as the sole carbon source. Thus, only these two (2) individual PAHs were further used for enrichment and degradation studies.

4.2.6 Enrichment and isolation of the bacteria

In the enrichment process, 10 ml of the culture was mixed with a new 90 ml MSM medium in a conical flask with pre- dried fluoranthene or pyrene. The mixture was then continuously shaken for seven (7) days. On the seventh day, 10 ml of the mixture was taken out and mixed in a new 250 ml conical flask which was also pre-treated with fluoranthene or pyrene. The same steps were repeated for 5 times. At the end of the enrichment process, bacterial strains in the culture were isolated by spreading the 10- fold, serially diluted consortium on agar plates coated with a layer of fluoranthene or pyrene on the surface. The dispersion was done using a glass spreader. The plates were then incubated at 35 °C for 24 hours. Bacteria colonies that produced clear zones were picked up from the plates and purified by repetitive streaking on nutrient agar plates. After 24 hours of incubation of the last streaked bacterial colonies, the pure cultures were then subjected to an identification step using a Biolog automated

system (Biolog Microstation System). The system was based on the MicroStation™ System/ Microlog User's Guide (Biolog Inc., 2009). The Biolog is an advanced device for identifying and characterizing microorganisms. Biolog's technology uses each microb abilities to use particular carbon source to produce a unique pattern for that microbe. Fig. 4.4 shows the Biolog instrument used for the identification process.

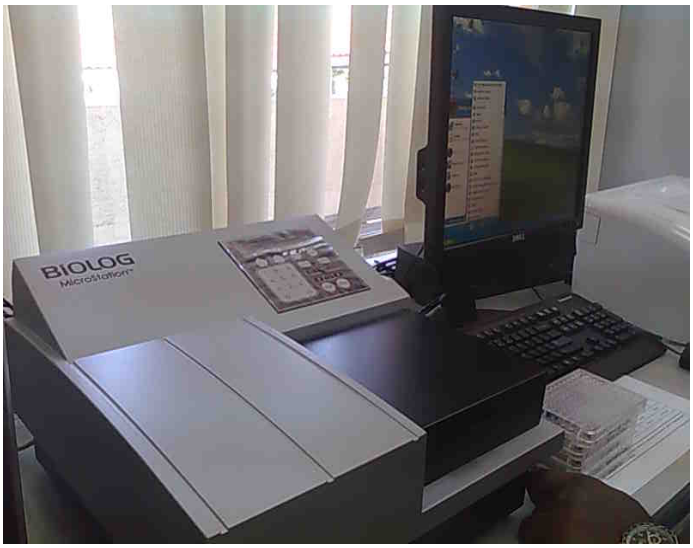

Figure 4.4 The Biolog machine

4.2.7 Preparation of the bacteria consortium

Only three (3) species of bacteria with the ability to degrade fluoranthene and three (3) species with the ability to degrade pyrene from both the contaminated road side soil and the termite fungal comb samples were selected to form the consortium. The fluoranthene-degrading bacteria from the contaminated road side soils were named RSS- F1, RSS- F2 and RSS- F3 while the pyrene- degrading bacteria were named RSS- P1, RSS- P2 and RSS- P3. Fluoranthene- degrading bacteria from the termite fungal comb were named T- F1, T- F2 and T- F3 while pyrene- degrading bacteria from the same source were named T- P1, T- P2 and T- P3.

To grow the bacterial consortium further for use in the degradation studies, three (3) colonies of each bacteria species were selected and inoculated in a conical flask with 100 ml of MSM medium that was pre- treated with fluoranthene or pyrene. The

mixtures were then subjected to the same enrichment steps for five (5) more times before they were used in the degradation studies.

4.2.8 Identification of bacteria species by using Biolog

Procedures for using Biolog were based on its MicroStation^{TM} System/ Microlog User's Guide (Biolog Inc., 2009).

Pure cultured samples prepared in 4.2.5 were subjected for inocula preparation. Since there was no clue at all on the species that will be obtained, Protocol A was chosen for use as it is suitable for identifying nearly all species of microorganisms. Growth colonies of bacteria on prepared pure culture were picked- up and mixed in an inoculation fluid (IF- A). The cell density of the mixture was ensured to be within 90-98 % as specified in the manual, to obtain good results.

The suspensions obtained were pipetted out and inoculated in GEN III MicroPlates which was designed for all aerobic bacteria. Each transfer was done within 20 minutes as long wait might cause inaccurate identification. Each well on the MicroPlate were filled up with exactly 100 µl. Sterile technique and pre- caution action were very important in each step to avoid any contamination which would fail the identification process. As soon as the suspension was dispensed in the MicroPlate, the MicroPlate was then incubated at 33 °C for up to 22 hours.

During incubation period, the microorganism will begin to use the carbon sources in certain well of the micro plate for respiration. For bacteria, this respiration process will reduce a tetrazolium dye and the well will change to purple color (Biolog Inc., 2001). After suitable incubation time was completed, the MicroPlate was read by using the MicroStation Reader. When the Reader read the pattern formed in the wells and searched the database, the bacteria would then be identified. The process would take only a few seconds.

4.2.9 Degradation study

The degradation of PAHs was performed by allowing the bacteria consortium to utilize a single PAH as the sole source of both carbon and energy. This degradation study, performed in a liquid culture, was prepared by adding 10 ml of 500 mg L^{-1} single PAH, diluted in acetone, into an empty and sterilized 250 ml flask to obtain a PAH solution with a concentration of 50 mg L^{-1}. The acetone was then evaporated by gently shaking the flask. 90 ml of MSM medium was subsequently added to each flask with the dried PAH. 10 ml of enriched bacteria consortium prepared previously were inoculated into the flask and the cultures were then continuously incubated and shaken in an incubator shaker at 150 rpm and 35 °C (as showed in Fig. 4.5).

In this degradation study, three (3) different conditions were tested. (i) Medium + PAH + bacterial consortium, (ii) medium + PAH, and (iii) medium + bacterial consortium, with (ii) and (iii) serving as controls. All treatments including the controls were in duplicates. 10 ml of the cultures were taken out every week on the seventh day for a month and were extracted with 3 × 10 ml DCM using a separation funnel (liquid-liquid extraction), concentrated and injected into gas chromatography- mass spectroscopic (GC- MS). All steps in the degradation studies were repeated with PAHs that were at 100 mg L^{-1} and 150 mg L^{-1} concentrations.

Figure 4.5 Condition of the cultures in the incubator shaker

4.2.10 Liquid- liquid extraction

Specifically, extraction of the samples was carried out by first pouring 10 ml of the collected sample into a 1 l separatory funnel. 10 ml solvent (DCM) was then added to the funnel and shaken for two minutes with periodic venting to release excess pressure. The organic layer was then allowed to separate from the water phase for a minimum of 10 minutes. The extract (top layer) was then collected in a 250 ml flat bottom flask.

A second 10 ml volume of DCM was then added to the sample bottle and the extraction was repeated for a second time, the extracts were then combined in the flat bottom flask. A third extraction was repeated in the same manner.

The total extract obtained was then concentrated to 200 µl by using rotary evaporator at 40 °C under reduced pressure, and transferred into a 2 ml Teflon- lined vial for GC- MS analysis.

4.2.11 Instrumental analysis

Gas chromatography- mass spectrometry (GC- MS) and it conditions used in this study was the same as described in Chapter 2 (Section 2.2.8). However, instead of SIM mode, this study was conducted in Full Scan data acquisition mode. The scanning was done from 50- 550 amu at 1.5 sec/ scan.

4.2.12 Quality assurance

GC reproducibility, procedural blanks, recoveries, sample duplicates were routinely analyzed with the samples and all data were blank corrected.

4.2.12.1 Reproducibility

The reproducibility for the gas chromatographic analysis for this study was examined by three injections of each PAHs standard used. The results are shown in Table 4.2. The reproducibility of the chromatographic procedure was measured by performing three injections in different days.

Table 4.2 GC- MS Instrument reproducibility in standard deviation (R.S.D.) percentage (%) for this PAHs study

Compounds	R..S.D. (%), n= 3
Acenaphthene	1.27
Fluorene	2.13
Fluoranthene	1.04
Pyrene	2.32
Chrysene	1.28
Benzo[a]pyrene	1.11
Indeno[1,2,3- cd]pyrene	3.65
Dibenz[a,h]anthracene	8.31
Benzo[g,h,i]perylene	2.14

4.2.12.2 Procedural blanks

To test the procedural blanks, pre- cleaned flasks and Petri dish with only MSM medium that was free from inoculation of bacteria and PAHs were used. These blank samples were then subjected to degradation steps as described in 4.2.5 followed by GC-MS analysis. The experiment was repeated three times.

Solvent blanks procedures were also carried out from time to time in order to monitor the background of the GC- MS. As described in Chapter 2, this procedure involved mixing 450 ml DCM and 50 ml of n- hexane and concentrated to 100 µl before being injected into GC- MS. The peaks were quantified and the amounts were subtracted whenever they appeared in the real samples so that the actual concentration of the compounds of interest could be obtained.

4.2.12.3 Detection limits

4.2.12.3.1 Instrument detection limit (IDL)

The GC- MS IDL was measured in this study was following method described in Chapter 2 (Section 2.2.9.3.1). The IDLs were measured by multiplying the standard deviation of the instrument responses with 3. Results of the detection limit are shown in Table 4.3.

Instrument Detection Limit (IDL) = Standard deviation x 3 Equation 4.1

Table 4.3 GC- MS Instrument detection limit (IDL) for this PAHs study

Compounds	IDL (mg L^{-1})
Acenaphthene	2.21
Fluorene	1.54
Fluoranthene	3.47
Pyrene	2.56
Chrysene	1.17
Benzo[a]pyrene	1.21
Indeno[1,2,3- cd]pyrene	3.24
Dibenz[a,h]anthracene	4.39
Benzo[g,h,i]perylene	4.27

4.2.12.3.2 Method detection limit (MDL)

The GC- MS MDL was measured by drying up or evaporates the solvent of 10 ml of 500 mg L^{-1} standard solution in pre- cleaned flask before adding the blank (blank MSM medium) and run it through step 4.2.6. This procedure was repeated 3 times and the mean value was used to calculate the MDL according to below equation:

Method Detection Limit (MDL) = s x 3.14 Equation 4.2

Table 4.4 GC- MS Method detection limit (MDL) for this PAHs study

Compounds	MDL (mg L^{-1})
Acenaphthene	0.32
Fluorene	0.22
Fluoranthene	0.24
Pyrene	0.37
Chrysene	0.31
Benzo[a]pyrene	0.17
Indeno[1,2,3- cd]pyrene	0.25
Dibenz[a,h]anthracene	0.13
Benzo[g,h,i]perylene	0.30

4.2.12.4 Recovery studies

Recovery studies were also carried out by drying 10 ml of 500 mg L^{-1} standard solution in pre- cleaned flask before adding the blank and run it through step 4.2.6. Results of the recovery studies are shown in Table 4.5. From the results, it can be seen that the

recovery are not high but it is still in an acceptable range and increasing week does not leave any significant difference towards the recovery values.

Table 4.5 Results of multi- step recoveries

Compounds	% Recovery ($\bar{x}$ ± R.S.D, n= 3)			
	Week 1	**Week 2**	**Week 3**	**Week 4**
Acenaphthene	65.1 ± 9.2	55.2 ± 11.6	59.6 ± 17.9	54.5 ± 16.4
Fluorene	51.5 ± 12.5	45.7 ± 15.2	56.1 ± 14.6	63.6 ± 12.9
Fluoranthene	64.2 ± 21.3	68.1 ± 13.4	51.6 ± 9.1	61.4 ± 25.4
Pyrene	61.9 ± 11.7	62.6 ± 10.9	62.8 ± 12.9	69.3 ± 11.3
Chrysene	51.7 ± 18.1	66.3 ± 21.6	60.9 ± 5.0	50.4 ± 16.4
Benzo[a]pyrene	68.4 ± 18.4	56.7 ± 18.1	53.7 ± 15.7	64.3 ± 11.9
Indeno[1,2,3- cd]pyrene	52.8 ± 21.6	57.6 ± 14.8	56.8 ± 12.4	59.1 ± 17.1
Dibenz[a,h]anthracene	69.3 ± 15.2	69.9 ± 19.0	56.1 ± 12.9	59.2 ± 15.6
Benzo[g,h,i]perylene	63.6 ± 17.6	65.8 ± 14.6	55.4 ± 14.2	54.3 ± 12.9

4.3 Results and discussion

4.3.1 Identification of the PAH- degrading strains

The Programmed Microbial Identification System built by Biolog has been proven to be useful in characterizing and classifying bacteria (Spiegelman *et al.*, 2005). Therefore, in this study, the species were identified using the Biolog Microbial identification system. The identification results are presented in Table 4.6.

Table 4.6 Identification of the bacteria from either the termite fungal comb or the contaminated road side soil

Samples	Source of Carbon	Organism	Identity
Termite fungal comb	fluoranthene	T- F1	*Ochrobactrum intermedium*
		T- F2	*Pseudomonas aeruginosa*
		T- F3	*Pseudomonas putida biotype B*
	pyrene	T- P1	*Pseudomonas putida biotype B*
		T- P2	*Ochrobactrum tritici*
		T- P3	*Pseudomonas aeruginosa*
Contaminated road side soils	fluoranthene	RSS- F1	*Ralstonia pickettii*
		RSS- F2	*Burkholderia cepacia*
		RSS- F3	*Pseudomonas resinovorans*
	pyrene	RSS- P1	*Ralstonia pickettii*
		RSS- P2	*Ochrabactrum anthropi*
		RSS- P3	*Corynebacterium appendicis*

Based on the identification results, two different bacteria genus that could degrade fluoranthene and pyrene were identified from the termite fungal comb. These genuses were *Ochrobactrum* and *Pseudomonas. Pseudomonas aeruginosa* and *Pseudomonas putida biotype B* were found to be capable of degrading fluoranthene and pyrene, respectively. *Ochrobactrum* species, in particular, *O. intermedium* and *O. tritici* could also degrade fluoranthene and pyrene respectively. *Pseudomonas* came from phylum *Proteobacteria*, class *Gammaproteobacteria*, order *Pseudomonadales* and family *Pseudomonadaceae* while *Ochrobactrum* came from the phylum *Proteobacteria*, class *Alphaproteobacteria*, order *Rhizobiales* and family *Brucellaceae*.

Ralstonia pickettii from the contaminated road side soil was found to degrade both fluoranthene and pyrene while *Burkholderia spp* and *Pseudomonas spp* from the same source degraded only fluoranthene. In contrast, *Ochrabactrum spp* and *Corynebacterium* isolated from the contaminated soil only degraded pyrene. *Ralstonia* came from phylum *Proteobacteria*, class *Betaproteobacteria*, order *Burkholderiales* and family *Burkholderiaceae* which was same as the taxonomy of bacteria from genus *Burkholderia.* Genus *Corynebacterium* came from phylum *Actinobacteria*, order *Actinomycetales*, suborder *Corynebacterineae* and family *Corynebacteriaceae*.

Several studies have been conducted to isolate, characterize and identify microorganisms with the capability to degrade a wide range of PAHs compounds (Weissenfels *et al.*, 1990; Yuan *et al.*, 2000; Dean- Ross *et al.*, 2001; Wong *et al.*, 2002; Prabhu and Pale, 2003; Santos *et al.*, 2007; Zhao *et al.*, 2009). *Pseudomon*as *spp*, a gram negative bacterium, has been reported as the main bacteria species that functions as a degrader of PAHs. They have been reported to be capable of degrading both low and high molecular weight PAHs if an appropriate growth medium is supplied (Yuan *et al.*, 2000; Juhasz and Naidu, 2000; Dean Ross *et al.*, 2001). Other than a PAH degrader, genus *Pseudomon*as has also been reported to have the capability to degrade other

organic recalcitrant pollutants. Although other genus of bacteria found in this study are not characterized as the main degrader of PAHs, previous researches have proved that the bacteria from genus *Ochrobactrum, Ralstonia, Bukholderia* and *Corynebacterium* are also capable and have a promising potential to degrade PAHs (Zhang, 2008; Ghosal *et al.*, 2010; Kim *et al.*, 2003; Chavez- Gomez *et al.*, 2003; Seo *et al.*, 2009; Othman *et al.*, 2011; Fernández- Luqueño *et al.*, 2011).

Although some of the bacteria used in this study have previously been identified as PAH degraders, it is believed that this is the first time that these bacteria were isolated from a different source (termite fungal comb) than in the previous studies to degrade PAHs. It is also believed that this is the first time that their capability to degrade PAHs in a consortium was identified.

4.3.2 Degradation studies

PAH researches in recent years focused more on the degradation of high molecular weight PAHs. The studies include the isolation and identification of several microorganisms that can mineralize and grow on four- ring PAHs when the PAHs are used as the sole carbon and energy source (Bouchez *et al.*, 1995; Boonchan *et al.*, 1998; Heitkamp *et al.*, 1988; Juhasz *et al.*, 1997; Ka¨stner *et al.*, 1994; Mueller *et al.*, 1990; Walter *et al.*, 1991; Weissenfels *et al.*, 1990). Some of these isolates have been used to identify the biochemical pathways involved in the catabolism of the PAHs.

In this study, degradation experiments were conducted using three different PAH concentrations: 50 mg L^{-1}, 100 mg L^{-1} and 150 mg L^{-1}. The degradation of PAHs was determined by calculating the remaining concentration of PAHs in the broth culture. An isolated bacteria consortium can only degrade PAHs in soluble form.

4.3.2.1 Degradation of fluoranthene by the bacteria consortium isolated from the termite fungal comb and the contaminated road side soil

Table 4.7 shows the reduction in concentation of fluoranthene due to its degradation by the bacteria consortium from the termite fungal comb and the bacteria consortium from the road side soil. The degradation trends are shown in Fig. 4.6 and 4.7.

Table 4.7 Degradation of fluoranthene by the bacteria consortiums isolated from termite fungal comb and road side soil

Bacteria's Sources	Time	50 mg L^{-1}		100 mg L^{-1}		150 mg L^{-1}	
		Remaining Conc. (mg L^{-1})	Reduction (%)	Remaining Conc. (mg L^{-1})	Reduction (%)	Remaining Conc. (mg L^{-1})	Reduction (%)
Termite fungal comb	Day 0	50.0 ± 0.00	0.00 ± 0.00	100.0 ± 0.00	0.00 ± 0.00	150.0 ± 0.00	0.00 ± 0.00
	Day 1	48.1 ± 3.07	0.44 ± 1.36	99.0 ± 5.17	0.21 ± 0.11	145.3 ± 110.02	0.90 ± 8.17
	Week 1	44.0 ± 2.78	8.32 ± 5.56	96.8 ± 2.03	2.47 ± 5.18	132.1 ± 193.00	9.79 ± 1.20
	Week 2	44.3 ± 3.90	8.83 ± 0.35	91.3 ± 7.42	8.01 ± 1.89	114.4 ± 52.41	21.90 ± 1.04
	Week 3	40.7 ± 3.23	15.61 ± 12.40	79.2 ± 8.24	20.20 ± 0.72	114.0 ± 155.23	22.61 ± 2.97
	Week 4	39.8 ± 3.13	17.63 ± 5.88	77.6 ± 7.77	21.80 ± 21.91	111.0 ± 153.12	24.30 ± 3.34
Road side soil	Day 0	50.0 ± 0.00	0.00 ± 0.00	100.0 ± 0.00	0.00 ± 0.00	150.0 ± 0.00	0.00 ± 0.00
	Day 1	48.4 ± 1.31	0.41 ± 0.11	98.7 ± 21.90	0.38 ± 0.08	147.2 ± 66.81	1.18 ± 1.45
	Week 1	46.9 ± 1.36	3.53 ± 1.00	90.1 ± 6.43	9.04 ± 9.21	113.1 ± 71.43	24.42 ± 22.71
	Week 2	46.8 ± 4.77	3.62 ± 1.06	86.8 ± 11.12	12.41 ± 11.22	109.2 ± 110.10	26.53 ± 26.70
	Week 3	43.8 ± 2.86	9.84 ± 2.22	72.7 ± 9.31	26.60 ± 3.40	101.1 ± 104.21	32.21 ± 0.83
	Week 4	35.0 ± 2.02	28.01 ± 16.21	67.2 ± 6.95	32.20 ± 3.33	95.0 ± 36.42	36.32 ± 14.01

Data are presented as mean ± standard deviations for the replicates.

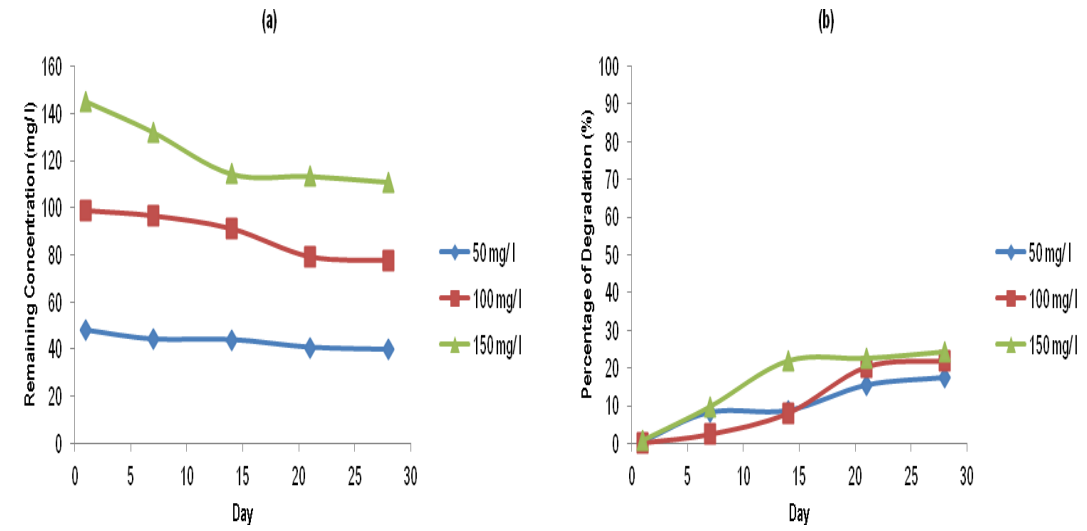

Figure 4.6 (a) The degradation trend of fluoranthene at three (3) different concentrations (mg L^{-1}). (b) The degradation trend of fluoranthene in percentage (%) by the bacterial consortium isolated from the termite fungal comb

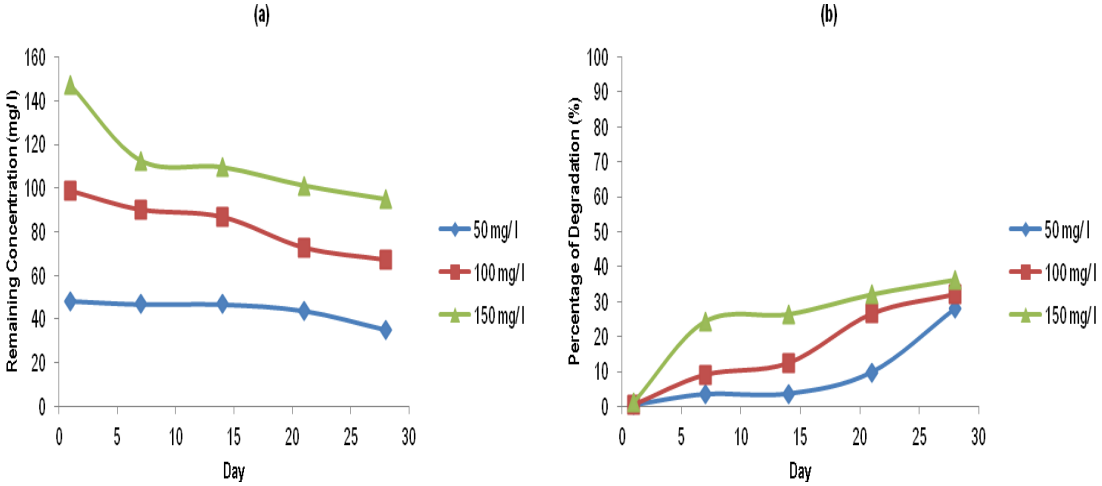

Figure 4.7 (a) The degradation trend of fluoranthene at three (3) different concentrations (mg L^{-1}). (b) The degradation trend of fluoranthene in percentage (%) by the bacteria consortium isolated from the contaminated road side soil

Table 4.7 as well as Fig. 4.6 and 4.7 demonstrate that the bacteria consortium isolated from the road side soil had a greater ability to degrade fluoranthene than the bacteria consortium from the termite fungal comb. When the bacterial consortium from the road side soil was used, 28 %, 32 % and 36 % total degradations of fluoranthene were measured for the 50 mg L^{-1}, 100 mg L^{-1} and 150 mg L^{-1} concentrations respectively. These were respectively 10 %, 10 % and 12 % greater than when the

bacterial consortium from the termite fungal comb were used in similar PAH concentrations. According to Zeng *et al.* (2010), fluoranthene placed in a liquid culture is harder to degrade than fluoranthene placed on a plate. In their study, only about 6 % and 12 % of fluoranthene samples (100 mg L^{-1}) were degraded in a 2- weeks incubation time by two (2) *Mycobacterium* strains namely NJS- 1 and NJSP. On the contrary, both bacteria degraded phenanthrene (100 mg L^{-1}) 100 % easily during the same incubation period. Zhou *et al.* (2008) in their study believes that there are some differences between the degradation in a liquid culture and on a plate.

4.3.2.2 Degradation of pyrene by the bacteria consortium isolated from the termite fungal comb and the contaminated road side soil

The degradation of pyrene by bacterial consortium isolated from both termite fungal comb and road side soil are detailed in Table 4.8, Fig. 4.8 and 4.9. Early studies on the bacteria degradation of pyrene were carried out by Heitkamp *et al.* (1988). From then on, various pyrene- degraders have been successfully isolated. Most of them belong to genus *Sphingomonas* (Leys *et al.*, 2004; Cunliffe and Kertesz, 2006), *Mycobacterium* and *Rhodococcus* (Miller *et al.*, 2004; Hennessee *et al.*, 2009). In this experiment, a combination of *Ralstonia pickettii, Ochrabactrum anthropi* and *Corynebacterium appendicis* as the bacteria consortium from the road side soil was identified to degrade pyrene better than the bacteria consortium from the termite fungal comb, which consisted of *Pseudomonas putida biotype B, Ochrobactrum tritici* and *Pseudomonas aeruginosa*. The differences in the total reduction between the pyrene degraded by the bacteria consortium from the road side soil and the pyrene degraded by the termite fungal comb at 50 mg L^{-1}, 100 mg L^{-1} and 150 mg L^{-1} concentrations were 4 %, 1 % and 3 %, respectively. These results show that the differences are small compared to the degradation of fluoranthene.

Table 4.8 Degradation of pyrene by the bacteria consortiums isolated from the termite fungal comb and the road side soil

Bacteria's Sources	Time	50 mg L^{-1}		100 mg L^{-1}		150 mg L^{-1}	
		Remaining Conc. (mg L^{-1})	Reduction (%)	Remaining Conc. (mg L^{-1})	Reduction (%)	Remaining Conc. (mg L^{-1})	Reduction (%)
Termite fungal comb	Day 0	50.0 ± 0.00	0.00 ± 0.00	100.0 ± 0.00	0.00 ± 0.00	150.0 ± 0.00	0.00 ± 0.00
	Day 1	47.3 ± 12.01	4.31 ± 1.09	96.9 ± 6.39	1.21 ± 3.21	138.1 ± 5.20	0.64 ± 4.67
	Week 1	47.1 ± 5.12	4.70 ± 5.15	70.3 ± 5.53	28.32 ± 0.07	124.2 ± 5.17	11.12 ± 7.08
	Week 2	43.1 ± 4.33	12.71 ± 12.72	66.7 ± 3.52	32.01 ± 1.69	117.1 ± 5.90	16.01 ± 0.28
	Week 3	40.4 ± 28.21	18.21 ± 12.71	65.7 ± 16.62	33.04 ± 6.70	92.2 ± 5.29	33.62 ± 2.71
	Week 4	31.9 ± 3.77	35.43 ± 4.18	59.5 ± 8.17	39.41 ± 5.41	81.1 ± 1.11	41.60 ± 4.03
Road side soil	Day 0	50.0 ± 0.00	0.00 ± 0.00	100.0 ± 0.00	0.00 ± 0.00	150.0 ± 0.00	0.00 ± 0.00
	Day 1	49.4 ± 11.21	0.38 ± 0.09	96.6 ± 21.12	1.63 ± 3.45	148.4 ± 17.81	0.58 ± 7.16
	Week 1	47.6 ± 6.19	4.02 ± 0.53	84.6 ± 3.86	13.82 ± 0.65	120.1 ± 3.26	19.74 ± 0.53
	Week 2	42.4 ± 4.47	14.50 ± 1.81	80.2 ± 26.84	18.31 ± 6.11	93.1 ± 5.45	37.50 ± 2.69
	Week 3	41.3 ± 33.03	16.72 ± 1.13	61.1 ± 10.52	37.81 ± 7.17	86.3 ± 2.23	42.41 ± 0.09
	Week 4	30.2 ± 25.11	39.22 ± 3.27	58.8 ± 3.18	40.12 ± 19.71	82.2 ± 6.04	44.80 ± 3.29

Data are presented as mean ± standard deviations for the replicates.

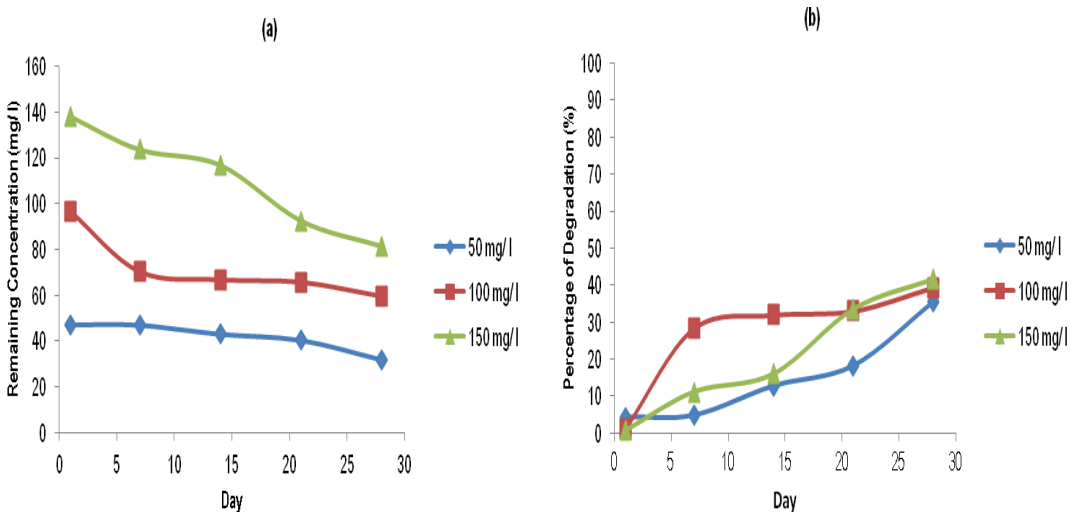

Figure 4.8 (a) The degradation trend of pyrene at three (3) different concentrations (mg L^{-1}). (b) The degradation trend of pyrene in percentage (%) by the bacterial consortium from the termite fungal comb

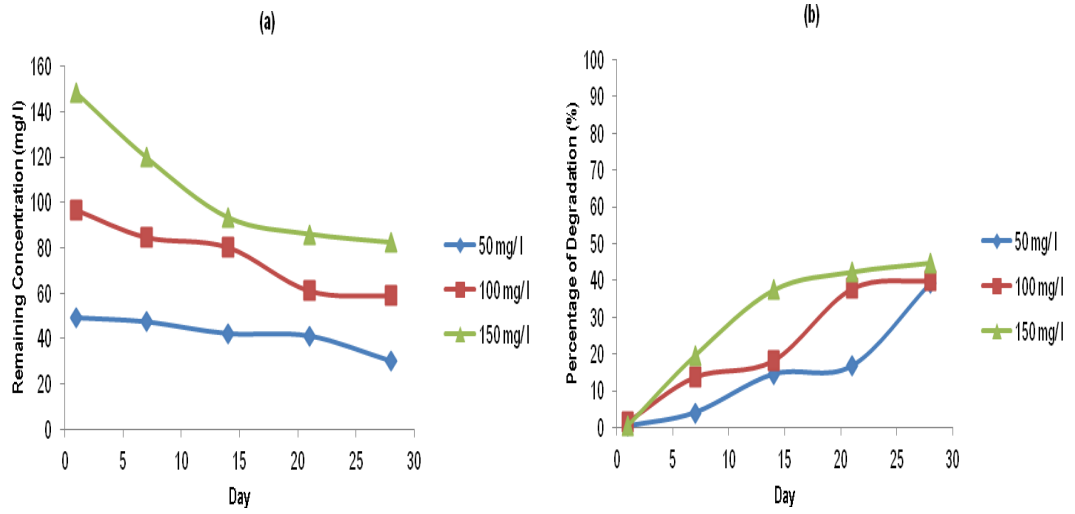

Figure 4.9 (a) The degradation trend of pyrene at three (3) different concentrations (mg L^{-1}). (b) The degradation trend of pyrene in percentage (%) by the bacterial consortium from the road side soil

From the degradation results of fluoranthene and pyrene, it can be seen that the bacteria combination of *Ralstonia pickettii*, *Ochrabactrum anthropi* and *Corynebacterium appendicis*, which were isolated from the road side soil, had a higher capability to degrade fluoranthene and pyrene than the bacteria consortium with the combination of

Pseudomonas putida biotype B, Ochrobactrum tritici and *Pseudomonas aeruginosa* that was isolated from the termite fungal comb.

The reduction in concentration of both fluoranthene and pyrene increased with time. However, the reductions recorded were poor since the measured total reductions for all samples were less than 50 % in the 4- week experiment. However, as the results obtained showed that the concentration of fluoranthene and pyrene were continuously reducing with time, it is believed that fluoranthene and pyrene can be degraded further if left for a longer period.

Moreover, it was identified from the studies that the degradation rate of both fluoranthene and pyrene increased with increasing PAH concentrations. This same situation was observed by Sayara *et al.*, (2009).

4.3.3 Degradation products

GC- MS analysis of the extracts obtained from the degraded fluoranthene and pyrene shows the existence of several degradation products (Fig. 4.10, 4.12, 4.15 and 4.19). ChemBioDraw Ultra 11.0 software was used to suggest the most possible names, chemical structures and other details of the degradation products based on their mass spectra (MS).

4.3.3.1 Degradation products of fluoranthene (degradation by the bacteria consortium isolated from the fungal termite comb)

Fig. 4.10 shows the degradation product of fluoranthene by the bacteria consortium from the termite fungal comb after 4 weeks in the conditions used in the experiment (incubated at 35 °C and shook at 150 rpm). From the figure, it can be seen that only one degradation product existed. The peak of the undegraded fluoranthene appeared at a retention time of 19.80 min. It was confirmed through the mass spectrum analysis of the fluoranthene where the molecular ion peak was at m/ z 202. In addition, the degradation product which existed

at retention time 20.20 min in the total ion chromatogram was identified to be 1-butyl-8-methyl-1,8a-dihydronaphthalene ($C_{15}H_{20}$, m/ z 200). This compound was determined based on its mass in the mass spectrum analysis while the structure was proposed based on the fragmentation pattern of its mass spectra as shown in Fig. 4.11. This compound was consistently found in all the 50 mg L^{-1}, 100 mg L^{-1} and 150 mg L^{-1} concentrations tested. The peak area of the product increased slowly with time and with decreasing fluoranthene's peak area. This suggests that the concentration of the product will increase if the concentration of the parent compound is decreased.

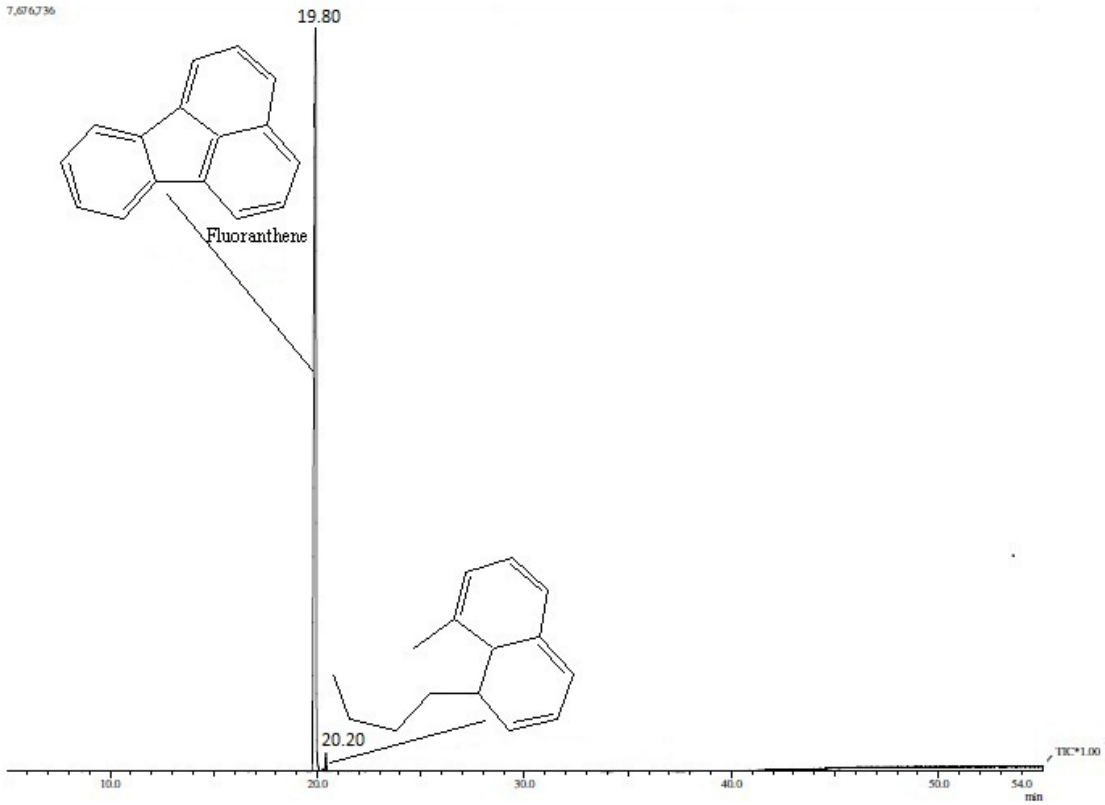

Figure 4.10 The degradation of fluoranthene by the bacteria consortium isolated from the termite fungal comb. Identified degradation product: **1-butyl-8-methyl-1,8a-dihydronaphthalene** (RT:20.20)

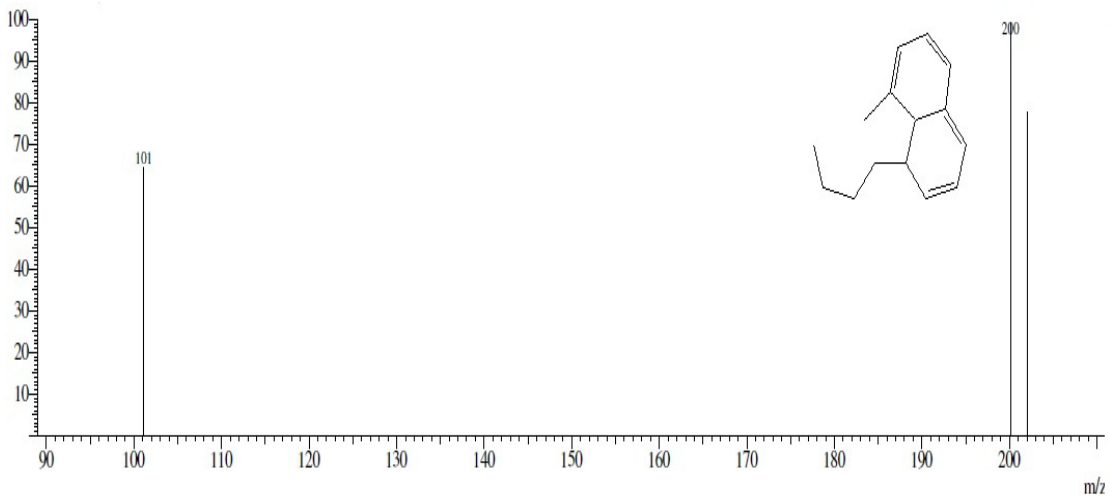

Figure 4.11 MS for 1-butyl-8-methyl-1,8a-dihydronaphthalene (Molecular weight:200)

To date, there have been no reports on the direct impact of 1-butyl-8-methyl-1,8a-dihydronaphthalene on the environment. Therefore, further studies are required to identify whether this compound are more or less toxic than its parent compound.

4.3.3.2 Degradation products of fluoranthene (degradation by the bacteria consortium isolated from the contaminated road side soils)

Based on their mass on the mass spectrum result (Fig. 4.12), two compounds were identified as the degradation products of fluoranthene when the bacteria consortium that was isolated from the contaminated road side soil was used. The peaks of the degradation products were found at 16.70 min and 20.90 min. These peaks represent 1-vinyl-9H-fluorene ($C_{15}H_{12}$, m/ z 192) and 9-methylene-1-vinyl-9H-fluorene ($C_{16}H_{12}$, m/ z 204) respectively. Their structures were derived based on their mass spectra and are shown in Fig. 4.13 and 4.14.

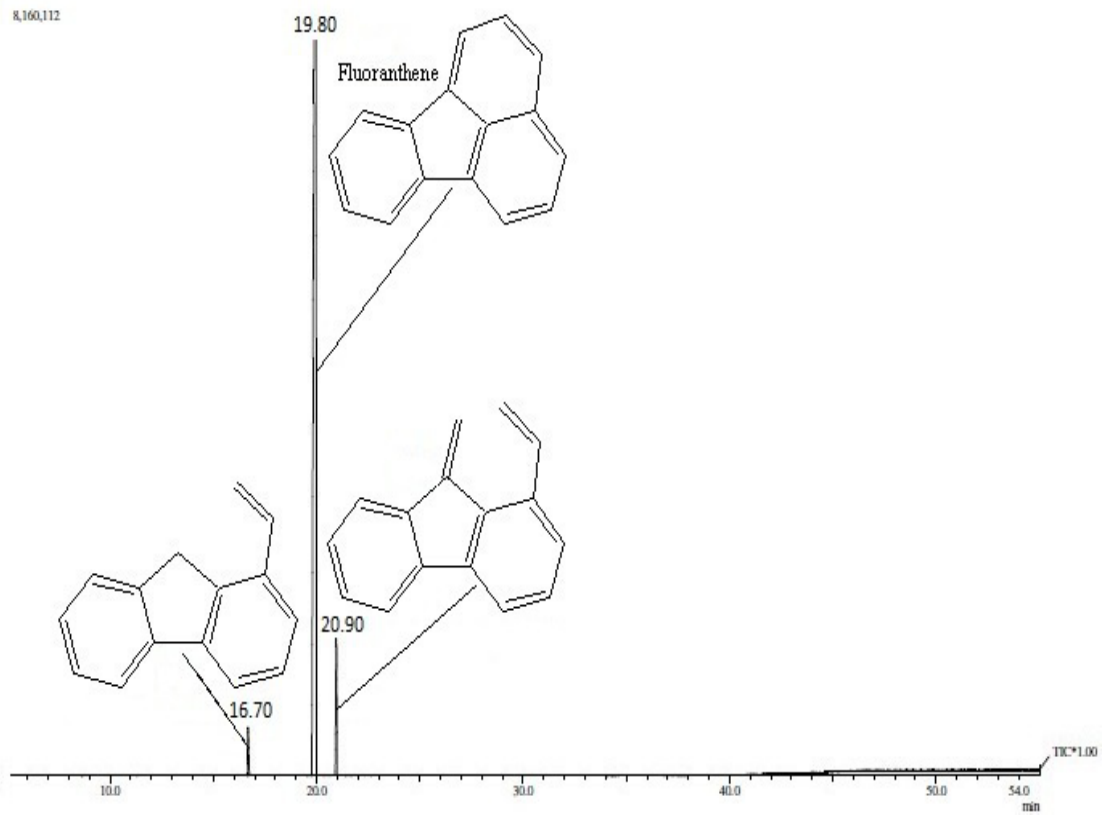

Figure 4.12 The degradation of fluoranthene by the bacteria consortium isolated from the contaminated road side soil. Degradation product: 1) **1-vinyl-9H-fluorene** (RT: 16.70) and 2) **9-methylene-1-vinyl-9H-fluorene** (RT: 20.90)

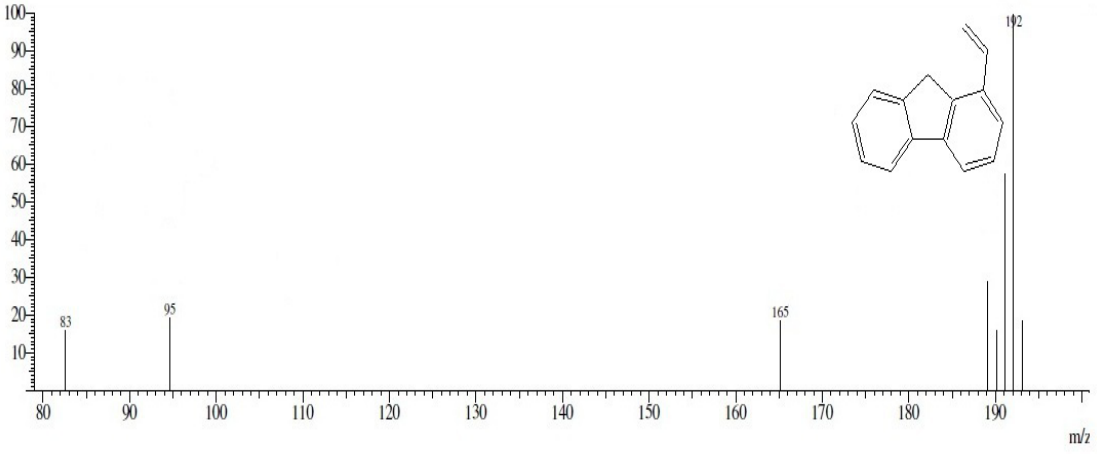

Figure 4.13 MS for 1-vinyl-9H-fluorene (Molecular weight:192)

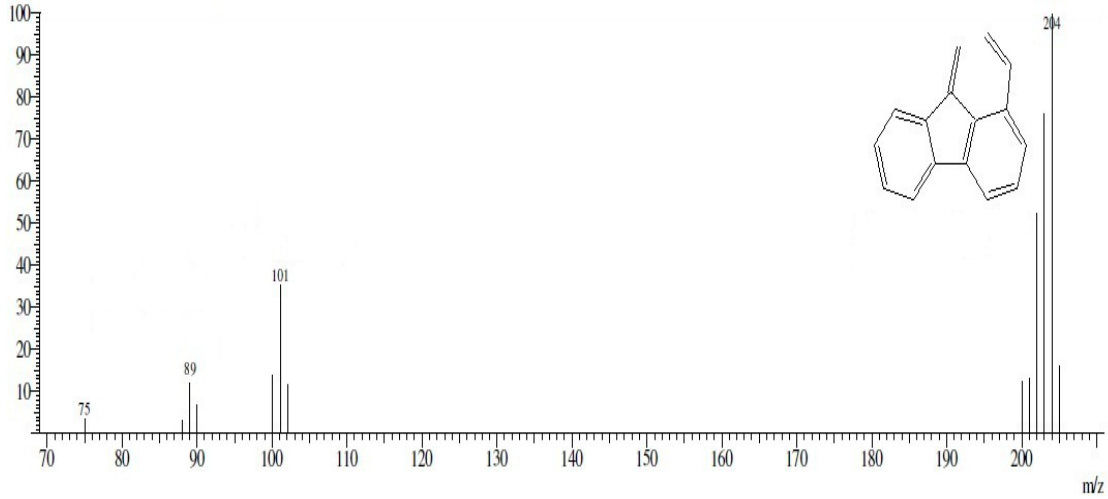

Figure 4.14 MS for 9-methylene-1-vinyl-9H-fluorene (Molecular weight:204)

More 9-methylene-1-vinyl-9H-fluorene was found to be produced than 1-vinyl-9H-fluorene after the 4th week. Unlike 9-methylene-1-vinyl-9H-fluorene that was consistently identified in all the concentrations studied (50 mg L^{-1}, 100 mg L^{-1} and 150 mg L^{-1}), 1-vinyl-9H-fluorene was not detected in the 1st week of observation for the 50 mg L^{-1} concentration and was only detected starting from the 2nd week. However, for the 100 mg L^{-1} and 150 mg L^{-1} concentrations of fluoranthene, 1-vinyl-9H-fluorene existed consistently and the peak area detected was found to increase slowly with time and with decreasing concentration of the parent compound.

4.3.3.3 The degradation products of pyrene (degradation by the bacteria consortium isolated from the termite fungal comb)

Fig. 4.15 shows the degradation products of pyrene when it was degraded by the bacteria consortium isolated from the termite fungal comb. Three (3) compounds were identified to be the degradation products. Findings from this study suggest that the bacteria consortium isolated from the termite fungal comb degraded pyrene more effectively than fluoranthene.

From the figure, the peak at 19.20 min corresponds to 1,10a- dihydropyrene ($C_{16}H_{12}$) with the molecular ion peak appearing at m/ z 204. Meanwhile, the peaks at 20.40

min and 21.60 min correspond to 9-ethyl-1-methyl-1H-phenalene ($C_{16}H_{16}$, m/ z 208) and 2-ethyl-1-isopropyl-8-methyl-1,2,3,4,4a,8a-hexahydronaphthalene ($C_{16}H_{26}$, m/ z 218) respectively. The structures of the three degradation products were derived from their fragmentation patterns in the mass spectrum shown in Fig. 4.16, 4.17 and 4.18.

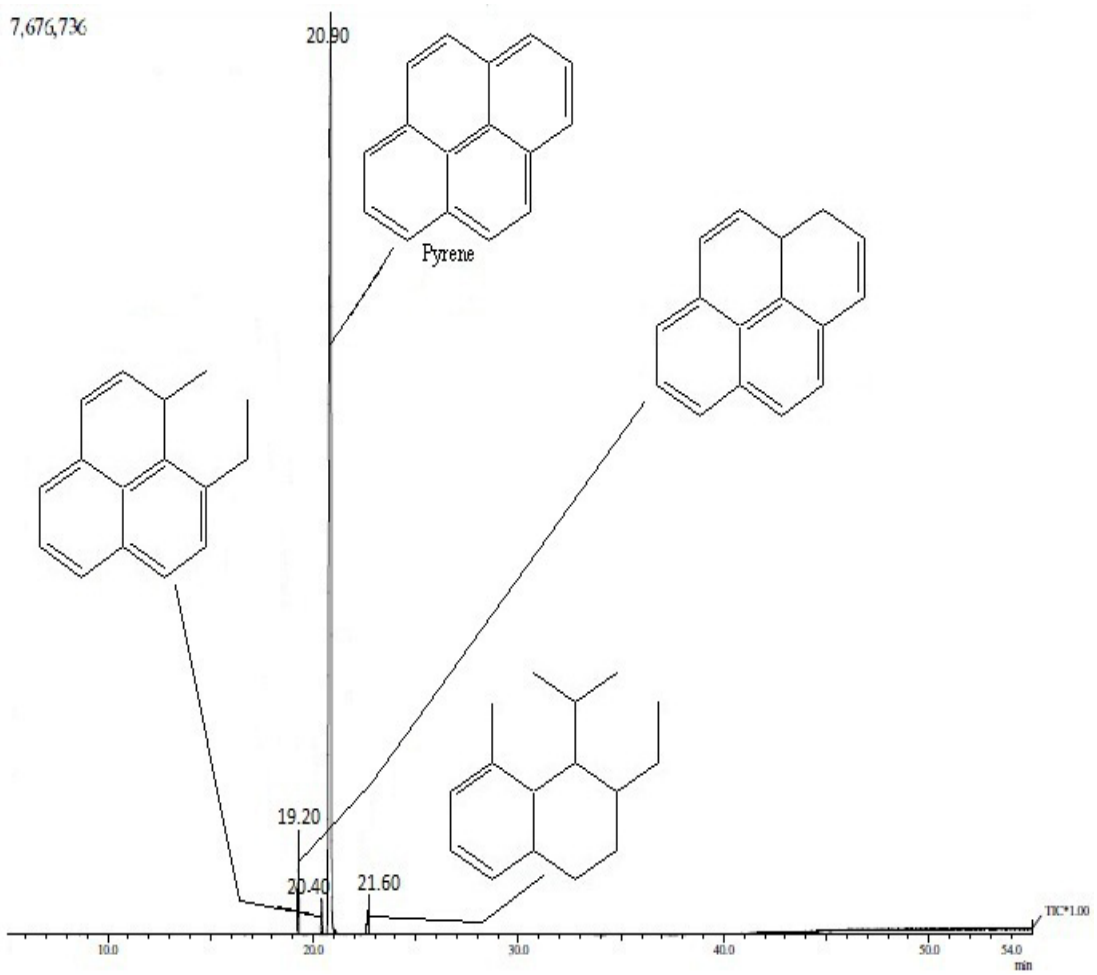

Figure 4.15 The degradation of pyrene by the bacteria consortium isolated from the fungal termite comb. Identified degradation products: 1) **1,10a- dihydropyrene** (RT: 19.20), 2) **9-ethyl-1-methyl-1H-phenalene** (RT: 20.40), and 3) **2-ethyl-1-isopropyl-8-methyl-1,2,3,4,4a,8a-hexahydronaphthalene** (RT: 21.60)

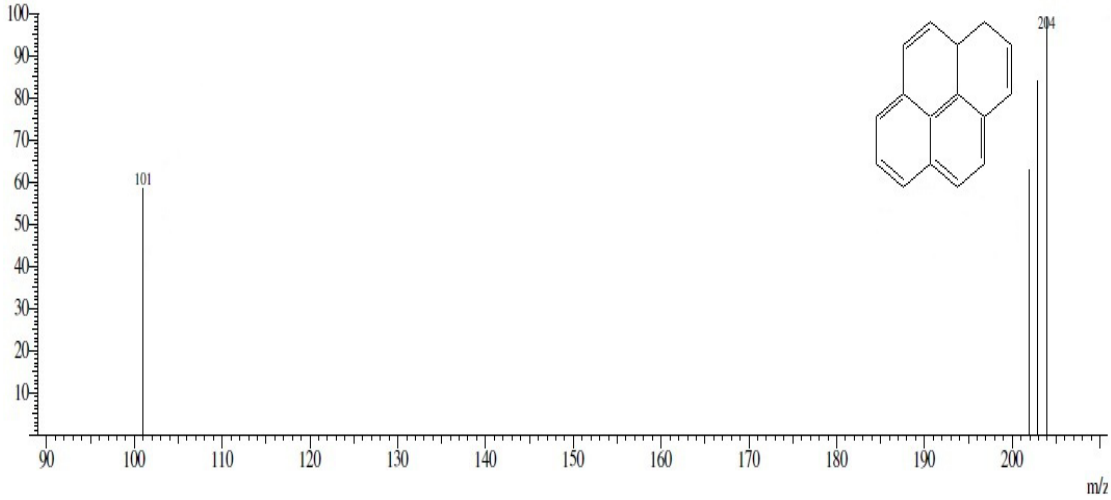

Figure 4.16 MS for 1,10a- dihydropyrene (Molecular weight:204)

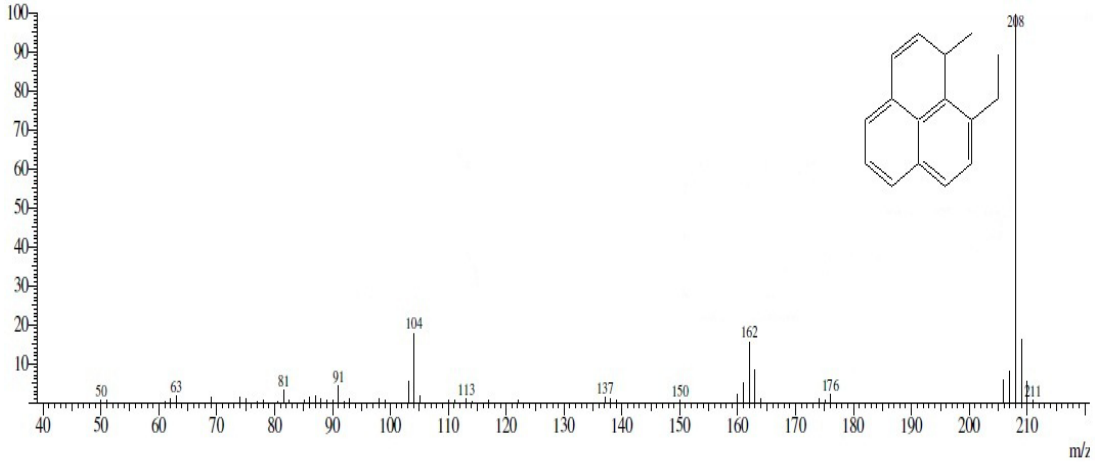

Figure 4.17 MS for 9-ethyl-1-methyl-1H-phenalene (Molecular weight:208)

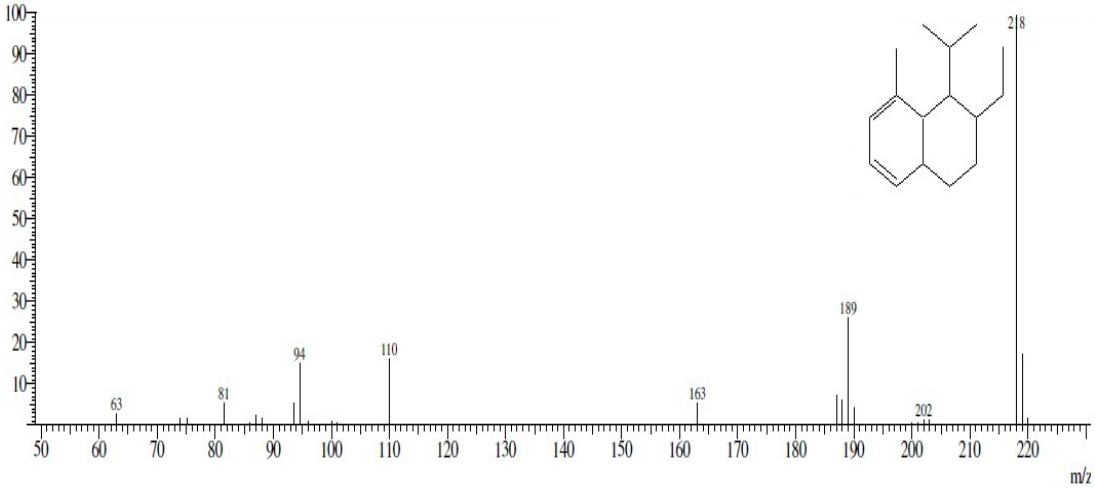

Figure 4.18 MS for 2-ethyl-1-isopropyl-8-methyl-1,2,3,4,4a,8a-hexahydronaphthalene (Molecular weight: 218)

All these three degradation products of pyrene were detected in all the three concentrations from the 1st week of the degradation studies. By the end of the 4th week, the peak area of 1,10a- dihydropyrene was found to be about two times higher than the peak areas of 9-ethyl-1-methyl-1H-phenalene and 2-ethyl-1-isopropyl-8-methyl-1,2,3,4,4a,8a-hexahydronaphthalene.

4.3.3.4 The degradation products of pyrene (degradation by bacteria consortium isolated from the contaminated road side soils)

Fig. 4.19 shows the degradation products of pyrene when it was degraded by the bacteria consortium isolated from the contaminated road side soils. There were four (4) compounds identified. Three (3) of them were the same products produced in the degradation of pyrene by the bacterial consortium isolated from the termite fungal comb. These three products were 1,10a- dihydropyrene (peak at 19.20 min, $C_{16}H_{12}$, m/ z 204), 9-ethyl-1-methyl-1H-phenalene (peak at 20.40 min, $C_{16}H_{16}$, m/z 208) and 2-ethyl-1-isopropyl-8-methyl-1,2,3,4,4a,8a-hexahydronaphthalene (peak at 21.60 min, $C_{16}H_{26}$, m/ z 218). The fourth degradation product was 7-butyl-1-methyl-1,2-dihydronaphthalene ($C_{15}H_{20}$, m/ z 200) which appeared at 21.00 min. Its MS is shown in Fig. 4.20.

Figure 4.19 The degradation of pyrene by the bacteria consortium isolated from the contaminated road side soil. Identified degradation products: 1) **1,10a- dihydropyrene** (RT: 19.20), 2) **9-ethyl-1-methyl-1H-phenalene** (RT: 20.4), 3) **7-butyl-1-methyl-1,2-dihydronaphthalene** (RT: 21.00) and 4) **2-ethyl-1-isopropyl-8-methyl-1,2,3,4,4a,8a-hexahydronaphthalene** (RT: 21.60)

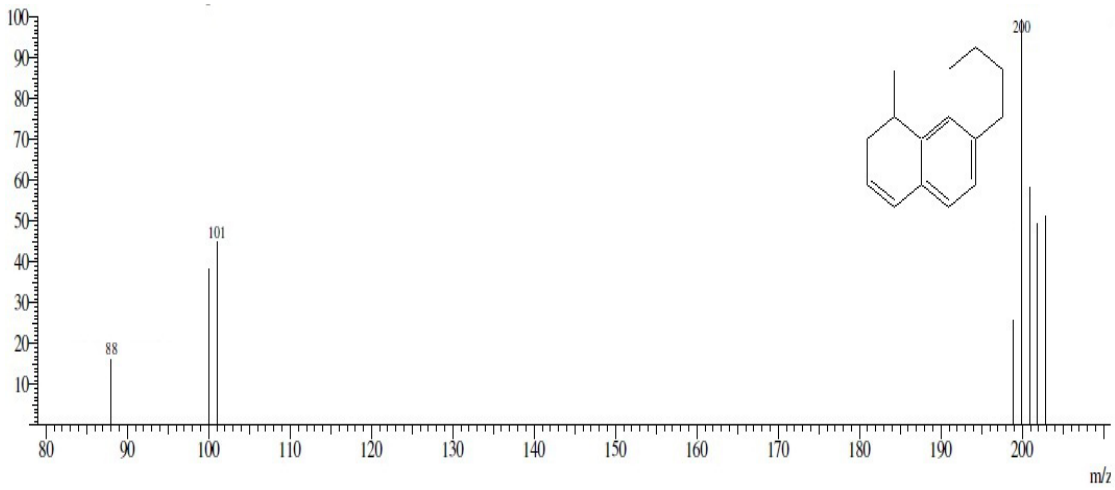

Figure 4.20 MS for 7-butyl-1-methyl-1,2-dihydronaphthalene (Molecular weight:200)

All the degradation products were detected from the 1st week of the experiment. The peak areas of 1,10a- dihydropyrene, 9-ethyl-1-methyl-1H-phenalene and 7-butyl-1-methyl-1,2-dihydronaphthalene were found to be about the same at the end of the experiment. In comparison, the peak area of 2-ethyl-1-isopropyl-8-methyl-1,2,3,4,4a,8a-hexahydronaphthalene was lower than the other products.

4.4 Conclusion

In conclusion, the results obtained from this study show that the isolation and identification processes had successfully identified several bacteria that have the potential to degrade PAHs. The bacteria were isolated from a termite fungal comb sample and a contaminated road side soil sample.Four (4) consortiums of microbes were found to be able to degrade PAHs (fluoranthene and pyrene), each consisting of three different bacteria. Two of the bacterial consortiums were isolated from the termite fungal comb while two others were isolated from the contaminated road side soil. With measured degradation percentage of less than 50 %, the degradation process induced by all the consortiums is considered slow. Several degradation products produced by degradation process were also identified but their toxicity in the environment remains uncertain.

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Chapter 5

Chapter 5

General Conclusion

The identification, concentration, sources, distribution and hazardous effects of PAHs in two recreational urban lakes located in Kuala Lumpur city were evaluated. This study was aimed to assess the impact of surrounding activities towards PAHs pollution in aquatic environment as well as to set up background value for PAHs in urban aquatic environment of Malaysia which is important for future surveys. The results obtained are also important as a reference in choosing the best disposal method for the sediments. In general, 15 primary PAHs which are listed by USEPA as primary pollutant have been identified in both lakes and their concentration vary from high in surface water and SPM and low in surface sediments in comparison to other studies worldwide. Sources of PAHs for both lakes are believed to come from both petrogenic and pyrogenic sources. Further evaluation on the sediments was done on their possible harmful effects towards living organism to determine options available for it disposal. The assessment was done by calculating the TEQ^{carc} of the surface sediments. From the result, it is believed that possible carcinogenic effects from the sediments are still too low and can be neglected. Thus, the sediments are safe to be used for agriculture and etc.

The PAHs concentration, distribution, and toxicity in three types of land use areas of Kuala Lumpur city were also determined and evaluated. This study involved road dusts and road side soils as the samples and sampling locations were selected based on the land use. Five industrial areas, six commercial areas and another six residential areas were selected in representing land use spots of Kuala Lumpur city. Generally, results from this study indicated that PAHs level in road dusts and road side soils of the studied industrial,

commercial and residential areas were found to be higher than literature values and guidelines established. Therefore, any agricultural activities in these areas are highly not recommended. Sources of the PAHs in road dust and road side soils of all sampling areas were believed to come from both petrogenic and pyrogenic activities. Risk assessment done based on the concentrations of PAH obtained indicated that carcinogenic risk from PAHs in road dusts and road side soils was still at “extremely low” level.

The capability of two bacteria consortium isolated from contaminated road side soil and termite fungal comb to degrade PAHs was also investigated. As levels of pollution worldwide increased tremendously year to year, many researcher works on finding any possible options to reduce it. PAHs were among the pollutants released in abundant into the environment and required huge attention for reduction. Instead of looking for new method in reducing the pollutant, identifying natural sources that can work in reducing PAHs are also important. In this study, bacteria consortia capable in degrading PAHs have been identified. However, the degradation performed was considered as slow since the degradation percentages in all experiments were less than 50%. A few degradation products have been generated from the study and the impact of these products in comparison to their parents compound are still unknown.

5.1 Future Work

Even though PAHs has been established as priority pollutant in countries like United States of America and United Kingdom, there is still no a guideline or action taken in enforcing it in Malaysia. However, as time goes by, the importance of studying PAHs became more crucial in Malaysia and the data on it became more important. Future studies on this subject are indeed necessary. These include:

1. Further monitoring of PAHs in Kuala Lumpur city by extending the coverage to all areas of Kuala Lumpur city. These will establish a complete background data bank for PAHs in Kuala Lumpur

2. Extending the monitoring to all part of Malaysia. This is important to assess pollutant (PAHs) level in Malaysia as well to establish a complete background data

3. Re- assessed PAHs level in the same location for a few years to study PAHs trend in Kuala Lumpur city

4. Identify more bacteria in the environment that are capable to degrade PAHs

5. Study on the application of the bacteria consortium for the remediation of contaminated soil.

Appendices

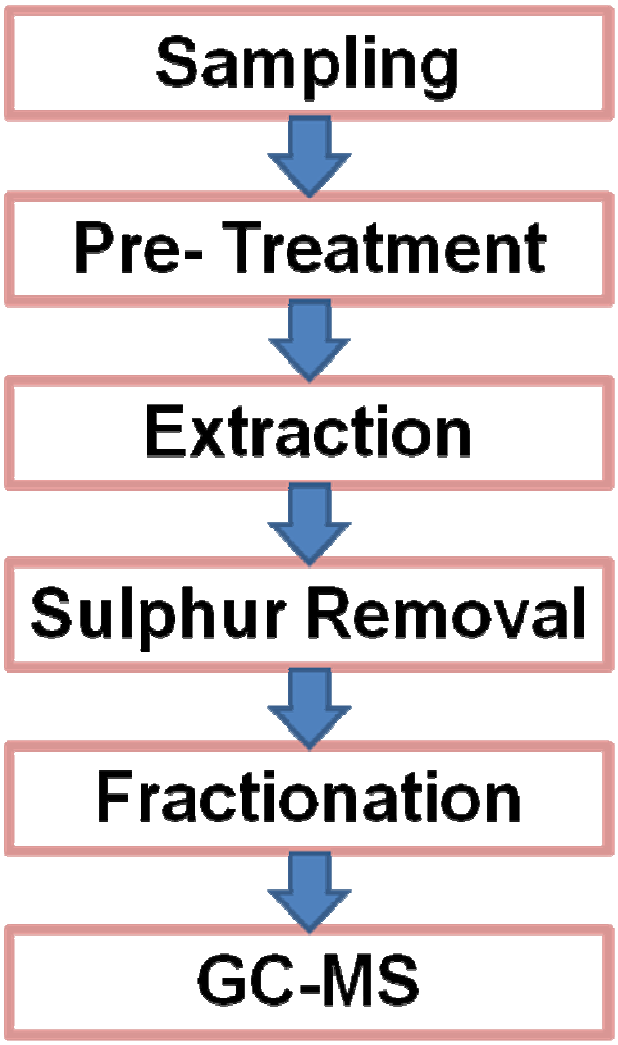

Figure A1 Schematic flow- chart for PAHs monitoring in water, SPM, surface sediments, road dusts and road side soils

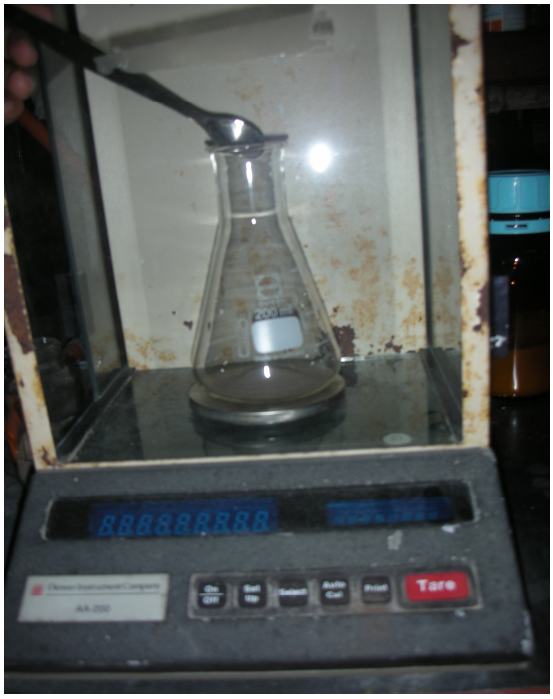

Figure A2 Sediments, road dust, and road side soil samples weighing using a top loading electronic balance

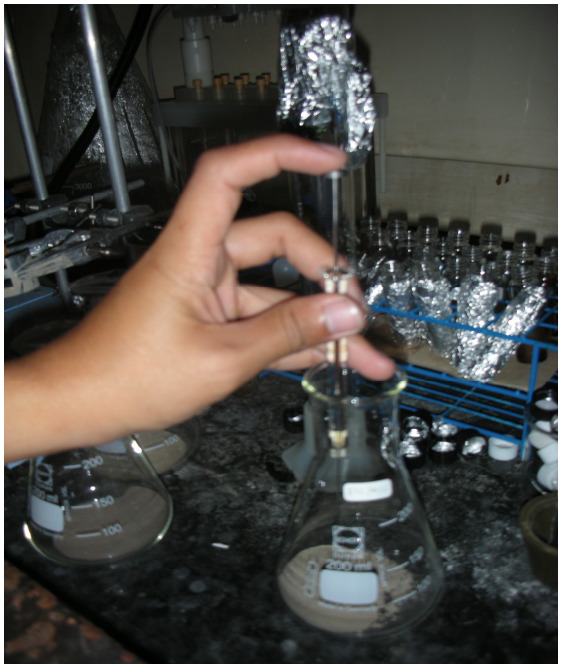

Figure A3 Standard solution injection

Figure A4 Extraction by ultra- sonification

Figure A5 Filtration process

Figure A6 Concentrating samples by using rotary- evaporator system of Buchi model (Switzerland)

Figure A7 Column chromatography

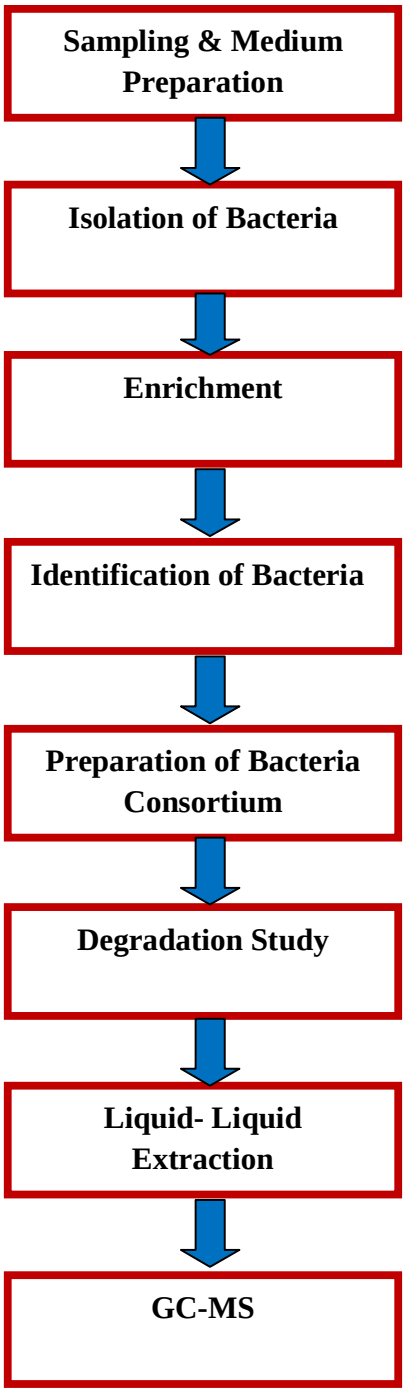

Figure A8 Schematic flow- chart for PAHs degradation study by using bacteria consortium isolated from termite fungal comb and contaminated road side soil

Figure A9 Termite fungal comb and road side soil samples weighing using a top loading electronic balance

Figure A10 Isolated samples

Figure A11 Spreading of isolated bacteria on MSM medium

Figure A12 Bacteria growth on fluoranthene

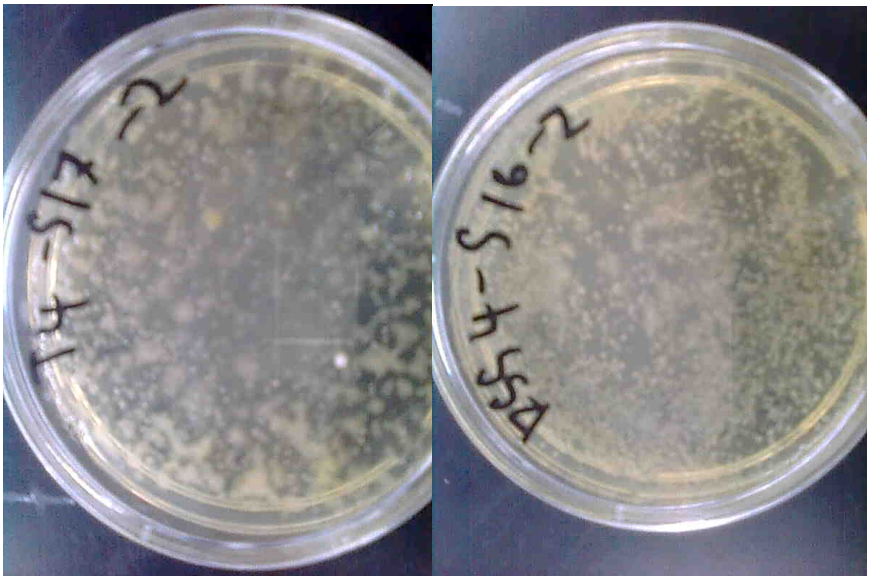

Figure A13 Bacteria growth on pyrene

Figure A14 Selection of bacteria for identification

Figure A15 Inoculation of bacteria in IF-A

Figure A16 Spreading of inoculated fluid into MicroPlates

Figure A17 Incubation of filled MicroPlates

Figure A18 Reading of bacteria species by Biolog

Figure A19 Degradation Studies in an incubated shaker

Figure A20 Liquid- Liquid Extraction